CENTRE FOR BIOPROCESS
CeBER
ENGINEERING RESEARCH

THE CENTRE FOR BIOPROCESS ENGINEERING RESEARCH

SHOWCASE

2018

UNIVERSITY OF CAPE TOWN
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CeBER SHOWCASE

FOREWORD

The Centre for Bioprocess Engineering Research (CeBER) at the University of Cape Town is active in converting biological concepts, metabolism and biomimicry opportunities into bioprocesses and products. The applications of these processes and products cover the spectrum from high value products – for health, nutrition, food and feed – and the fine chemicals industry – through commodity products, the biorefinery, waste processing and re-purposing – to mineral bioprocessing and pollution prevention. In this booklet we provide an overview of projects underway in 2018 in CeBER.

The researchers on these projects are a combination of MSc and PhD students, post-doctoral research fellows, research officers and academics. The research undertaken aims to contribute to our training of process engineers, applied scientists and bioprocess engineers for South Africa. CeBER strives to equip South Africa with generation of interdisciplinary knowledge relevant to its context in the field of bioprocess engineering in which we integrate a deep understanding of life science and engineering principles. I trust that you will find our work interesting.

Prof Sue Harrison
Director: CeBER

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MINERALS & BIOHYDROMETALLURGY



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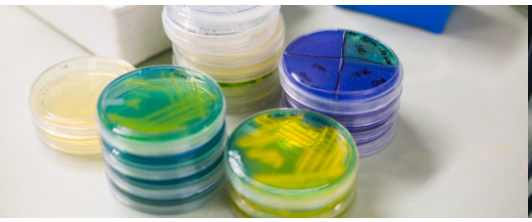


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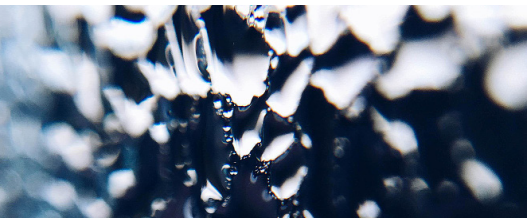

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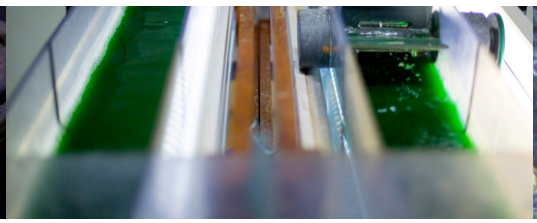
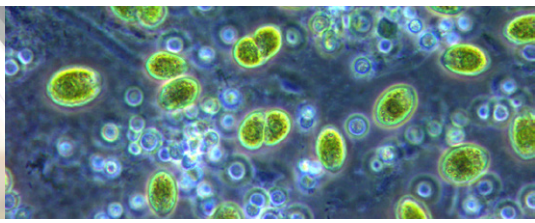
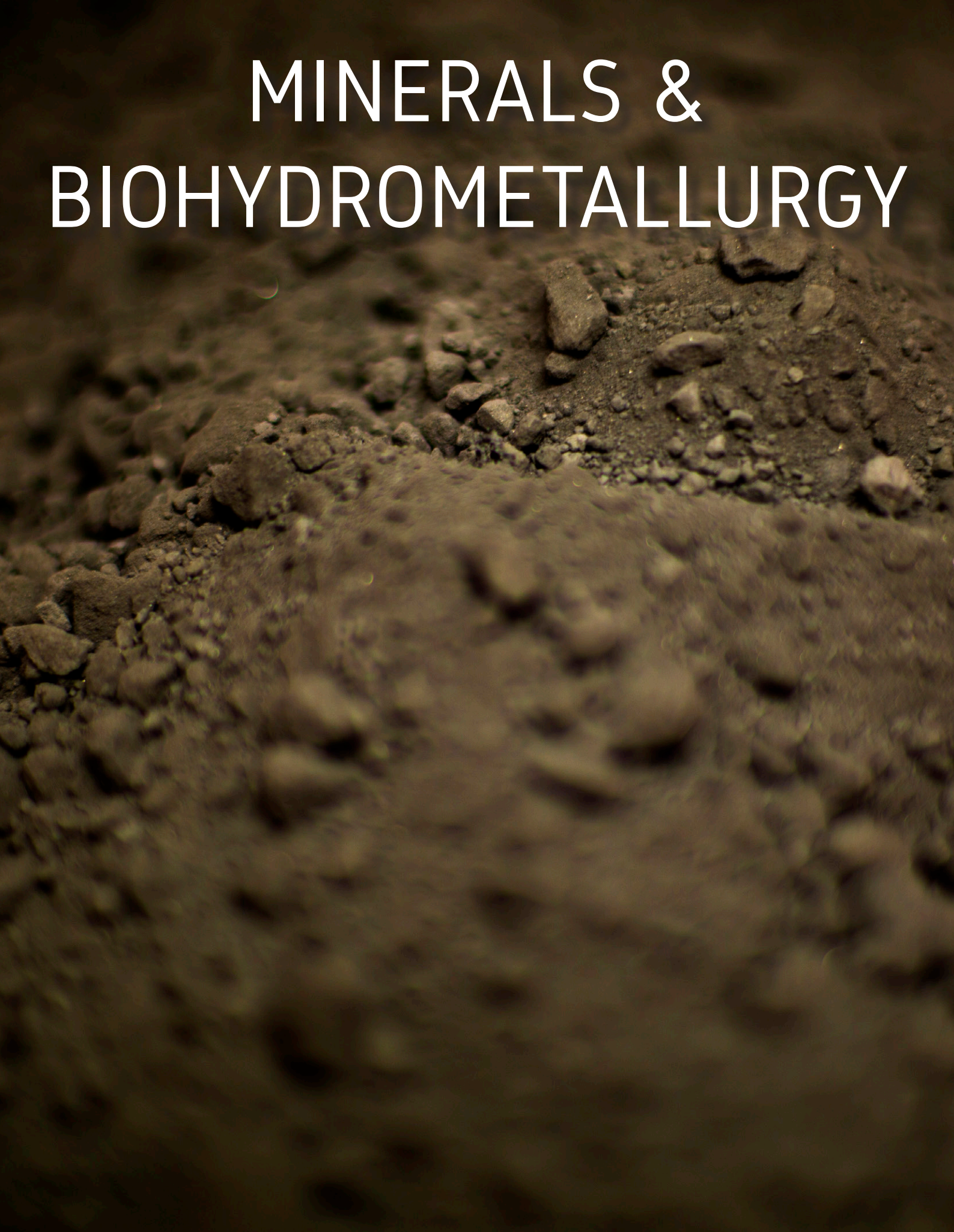



MINERALS & BIOHYDROMETALLURGY



The Semi-Continuous UCT Biokinetic Test Provides Improved Characterisation of Acid Rock Drainage Potential of Sulfidic Waste Rocks

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Keywords: acid mine drainage, microorganisms, neutralization rate, acid neutralizing capacity

Large quantities of waste rock are generated in the extraction of the base and precious metals and of coal; the ratio of waste to mineral off value depending on product value but increasing with resource depletion. Sulfide-bearing mineral wastes oxidize in the presence of water and air to produce acidic, sulfate-rich rock drainage (ARD). Where ARD is considered important, long-term kinetic tests determine the rate of the net acid generation under field conditions. However, the relative kinetics of acid neutralization and generation and the effect of micro-organisms are not considered in these tests. More recently, approaches to refine ARD characterization and prediction were developed. The UCT biokinetic test is one of a suite of tests informing this characterization. The UCT batch biokinetic test protocol provides insight into the rate of acid neutralization and acid generation in the presence of Fe & S-oxidising micro-organisms over 90 days. However, the batch biokinetic test limits the results to a net acidification level. Further, the easily soluble minerals with neutralising potential weathered in the initial stages of the test may affect the viability and hence effectiveness of microbial consortium used as inoculum in the test.

The kinetics of acid neutralizing and acid generating reactions in the presence of iron and sulfur-oxidizing micro-organisms are investigated, using waste rock from gold-bearing ores with high acid neutralising capacity (ANC) and copper-bearing ores with low ANC and carrying out both static tests and the draw and fill biokinetic test. The two waste rocks used were found to be potentially acid forming. The semi-continuous test provides a more rigorous approach to characterisation of ARD potential, owing to the slow washout of soluble neutralisation potential and oxidation products with time and the maintenance of conditions under which the naturally occurring acidophilic microorganisms thrive, thereby better representing the open-flow systems found in the field. Microbial species proliferating in these tests were monitored as leaching progressed and demonstrated the natural occurrence of key iron-oxidisers and sulfur-oxidisers such as L. ferriphilum and At. caldus on the waste rock. The data presented provide confirmatory and complementary results, demonstrating the differing sample characteristics and contributing to greater understanding of ARD generation potential of mine wastes.

Establishing the Flow-Through Biokinetic Test to Characterise Sulfidic Waste Rock Mineral for its Potential to form ARD

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Keywords: Acid rock drainage, waste rock surface, flow-through biokinetic test, colonisation

A major environmental problem facing the mineral industry and current and past mining regions harbouring sulfidic minerals is acid rock drainage (ARD), resulting from the oxidation of sulfidic mineral waste rock. Oxidation of the sulfidic mineral, especially pyrite (FeS2), in waste rock is caused by its exposure to both oxygen and water and is exacerbated by the presence of iron and sulfur oxidising microorganisms. Characterisation of acid rock drainage (ARD) generation potential of mine wastes, through small scale, short term tests, is critical for effective disposal or re-purposing of the wastes and thus prevention of ARD formation.

The current batch biokinetic test developed at UCT accounts for the role of microbial activity in ARD generation, not previously considered in the static tests and provides relative kinetic data collected over a 30-90 day period. It does not, however, represent the typical contacting mechanism for waste rock nor does it account for washout of neutralising capacity typical of a flow-through system such as the waste rock dump. Refinement of the batch biokinetic test into a flow-through test has potential to remove these limitations.

In this study, two pyritic waste rock samples, liberated by milling, were characterised using three biokinetic test approaches: the slurry batch test (BT), the batch test using mineral-coated beads (BT-CB) and flow-through column test with mineral-coated beads (FT-CB). Results have shown through static tests, solution redox potential and pH analysis that the two waste rocks were acid forming. Furthermore, it was demonstrated in the FT-CB system that microbial proliferation on the waste rock surfaces progressed with time and as a result, oxidative exothermic reactions facilitated by the increasing microbial presence on the surfaces was demonstrated using isothermal microcalorimetry. This study informs the on-going refinement of the biokinetic test through establishment of a flow-through test for ARD characterisation.

Development of an Integrated Approach for Acid Rock Drainage (ARD) Prediction from Waste Rock

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Keywords: Acid Rock Drainage

The generation of substantial quantities of waste material with little economic value is a direct consequence of the mining of metal-bearing ores. The problems arising from these wastes are numerous and multi-faceted. Of particular environmental concern is the contamination of water courses through the generation and transport of acidic rock drainage (ARD) from sulfidic mine wastes. This pollution often contains elevated concentrations of toxic metal anions and salinity concentrations, with ARD generation often exceeding the working life-time of the mining operations by decades and centuries. At source, prevention, containment and treatment remains the ideal strategy for the management of ARD. This, however, necessitates the accurate and reliable characterisation and prediction of the potential hazards associated with ARD generation. The current standard characterisation and prediction methods fail to account for a multitude of factors affecting ARD generation. Limitations in the physical, chemical and biological conditions used in the current test methods often fail to provide realistic estimates for ARD generation in the field. The consequence of these may lead to large-scale misclassification of mine wastes.

This study aims to improve the reliability of ARD characterisation and prediction through the development of an integrated framework to assess ARD generation at the laboratory scale. In conjunction with the current methods, improved test methodologies will be developed for ARD generating systems with time, recognising the importance of factors currently not accounted for. Integration of the experimental results with detailed knowledge from mineralogical and geochemical analysis will allow for a better understanding of the potential for pollution formation. Validation of the laboratory-scale results with those obtained from field-scale tests will be performed using mathematical modeling. Improvements in ARD characterisation and prediction will allow for the upfront implementation of management strategies for ARD generation during the working lifetime of the mining operation.

Fabricated Soils from Coal Waste

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Keywords: mine waste repurposing, rehabilitation, topsoil, technosols

Coal still plays a key role in the South African energy sector as extensive reserves of this resource are available. In the past few decades, efforts have focused on clean coal technologies and, in particular, end-of-pipe technologies such as carbon capture and storage, cleaner combustion technologies, and control of pollution using costly disposal and treatment facilities. Very little emphasis was placed on opportunities to reuse or recycle the downstream wastes. More recent studies are placing specific focus on developing skills and technologies for the prevention of environmental degradation and pollution using more preventive and proactive approaches, aimed at using coal wastes. For example, in the production of fabricated soils, coal waste management is may be achieved through the integration of appropriate wastes into the landscape by the establishment of a vegetative cover, thereby mitigating part of the environmental impact caused by final disposal.

In this project, the production of a topsoil from South African coal fines and ultrafine wastes is demonstrated. Using mixtures comprising of desulfurised and as received coal wastes, amended with other industrial and urban wastes, satisfactory conditions are provided to promote plant growth. In the first instance, this soil could be used in restoration and rehabilitation itself, avoiding the need for extraction of soil from borrow pits, which is a common practice in restoration activities.

Fundamental research is being developed to address key issues for soils derived from mine wastes, including: soil acidity and neutralisation reactions, macro- and micronutrient availability, organic matter content, microbial community deportment, the mobility of metals and phytotoxic compounds, and the physical structure of the soil. This study focuses first and foremost on the development of sustainable technologies with respect to coal waste final disposal challenges by considering innovative and long-term solutions for the re-purposing of waste material from coal processing, thereby addressing both long-term risk removal and value recovery.

Acid Bio-Desulphurisation of Coal Discards Using Heap Leaching Protocols

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Keywords: acid rock drainage; coal; bio-desulphurisation

Both coal and hard rock mining operations result in a large amount of waste rock and coal discards. These wastes are an environmental concern, especially where the exposure of sulphide minerals present in these discards to natural oxidants results in acidic runoff, referred to as acid rock drainage (ARD). Heap bioleaching is typically used as a means to extract base metals from low grade ores. Here we consider its potential in developing a prevention strategy for long-term ARD generation. Heap leaching has potential as an easily implementable and cost-effective approach for bio-desulphurisation.

Through the controlled initiation of the bioleaching reactions, the acid generated within these systems can be used to assist the coal desulphurisation process. The iron solubilized as ferrous iron is microbially oxidized to the leach agent ferric iron, facilitating further leaching. Consequently, leaching reactions are accelerated to produce an environmentally benign waste with economic prospects. The leachate is collected and further processed to minimize environmental burden and re-purpose components to products of value.

In this project, laboratory columns tests are used to mimic conditions in a typical heap and optimize heap operating conditions for coal desulphurisation. High sulphur coal discards from eMalahleni, Mpumalanga are treated and opportunities for re-purposing of the iron and sulphate rich leachate solution are explored. Pre- and post-leaching characterization of the coal discards show promise in application of heap leaching technology for re-purposing of the solid waste coal and for ARD prevention.

Development of Co-Disposal Methods in the Prevention of Acid Rock Drainage

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Key words: packing methods, drainage, compressibility, acid mine drainage

There is a growing need to develop post-closure mine reclamation practices that limit the exposure of the sulfidic waste to oxidation environments. Oxidative weathering of sulfides occurs in aqueous conditions in the presence of oxygen. Catalytic action of iron and sulfur oxidising microbes further propagate the generation of acidic lixiviates. To limit the environmental footprint, co-disposal of waste rock and desulfurised benign tailings has been explored. These waste materials have widely differing physical and chemical attributes, which complement each other in ARD mitigation endeavours. Waste rock matrices are relatively strong and incompressible. However, the presence of the higher sulfidic component and high permeability in such matrices gives rise to intensive oxidation processes. On the other hand, benign desulfurized tailings have lower hydraulic conductivity values and higher acid-neutralising potentials. To fully exploit these attributes, it is becoming increasingly crucial that engineered co-disposal methods are developed.

Conventional packing practices in bench-scale kinetic column tests conceal a variety of geotechnical issues. Preferential flow paths and rapid percolation rates prematurely promote the onset of ARD generating reactions. In this study, waste rock handling approaches are developed to impede the flow of oxidants through mine waste dumps both at laboratory and pilot scale. Waste rock to benign tailings ratios of 3:2 and 2:3 (w/w) were investigated. Hydrogeological properties, including void ratio, and slump tests, were investigated when developing packing procedures. As a measure of performance, compressibility tests were conducted to evaluate the structural integrity of the ore beds.

The compressive stress-strain-time response observed from these tests provided insight into the ideal packing methods thereby informing the structural set-up of waste beds in kinetic column tests of varying diameters. Effluent from the column tests was analysed for pH, redox potential, ferrous and total iron concentrations with time. Results indicated a relationship between load deformation, fluid flux reduction and acid rock drainage mitigation.

Can Fibre-Rich Plants Serve the Joint Role of Remediation of Degraded Mine Land and Fuelling of a Multi-Product Value Chain?

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Keywords: land remediation, transformation, fibre production, value chain

Over the last two decades, around 6000 mines have been abandoned in South Africa, resulting in a significant loss of economic activity in communities who are heavily dependent on jobs created by the mining industry. A community of practice (COP) project funded by the Department of Science (DST) and the National Research Foundation (NRF) is investigating the concept of mine transformation by proposing the use of land previously dedicated to mining for agricultural activities. Fibrous plants have been selected as the focal point of the study since they can simultaneously rehabilitate degraded mine land by absorbing metals from the soil, while their harvested fibres can be used for textiles and furniture, offering the possibility to develop a multi-product value chain.
The COP project takes an inter-disciplinary approach by tackling biological, environmental, legal, economic and social issues. For this purpose, CeBER is working jointly with the Minerals to Metals group, the Development Policy Research Unit (DPRU) group and the Mineral Law in Africa (MLiA) group, all based at UCT. CeBER's work is focussed on investigating the suitability of various fibrous plants for land remediation and fibre production. The research areas include the geographical features of potential sites and the soil type; the resilience of the plant and its productivity; the nutrient and water requirements. We are working closely with the Minerals to Metals group who are evaluating the downstream options for the recovery of metals and products from the fibrous plants. The DRPU is exploring the economic opportunities of fibrous plants by researching domestic and global markets for fibre-derived products. The work done by the MLiA group will facilitate the transition to a fibrous economy by identifying the adequate legal frameworks required for successful mine rehabilitation and transformation.

Reference:
University of Cape Town, 2018. *Towards Resilient Mining*

Conceptualisation, Selection and Transfer of Environmental Technologies in the Mining Industry: Moving from Labs Toward Real Life

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The world needs improved technologies and modes of consumption to be able to sustain life into the next millennium. This is evident in the mining industry where the non-regenerative nature of extraction and the concomitant environmental and social problems are becoming clear. Part of the solution is the development of environmental technologies to address some of these shortfalls. Universities and research organisations are working on developing such technologies, but novel technologies are not guaranteed acceptance and implementation in practice. This work developed an approach for early inclusion of industry in the conceptualisation, selection and transfer of such technologies with the aim of improving transfer strategy and technology uptake.

The case study for this work focused on opportunities for the re-purposing of separated sulfide-rich coal tailings and was based on the innovation value chain. Industry professionals were included in technology analysis during the selection phase and were asked to elucidate barriers and enablers that the technology implementation team is likely to face when embarking on technology transfer. The conceptualisation and selection approaches used in this study included identification and preliminary analysis of alternatives, multi-criteria performance assessment of selected alternatives, and a scenario analysis of the two preferred options to achieve a better understanding of the implications in the South African context. Industry professionals estimated the performance of the alternatives based on different criteria, thereby engaging with the technological alternatives early in the process.

This approach has illustrated the importance of including industry early in the development of novel technologies, in terms of technology focus but also in terms of understanding industry dynamics and questioning assumptions. For instance, researchers assumed that ideal transfer partners would be the mines themselves and hoped that the moral argument may carry some weight in transfer, both of which turned out not be the case

Fungal Assisted Bioleaching of Low Grade Minerals

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Keywords: Fungi, Bioleaching, Low grade minerals, Aspergillus, Penicillium, oxidic ores, carbonaceous ores.

Depletion of the high-grade mineral sources had given rise to a situation of urgency towards the development of a sustainable green technology for the effective and profitable recovery of valuable metals from their ores. A comprehensive study on the fungal bioleaching of valuable metals from low-grade and waste minerals has been conceptualized. Commonly, the process of bioleaching is based on oxidative processes facilitated by acidophilic microorganisms, as the metals of interest are in a reduced oxidation state housed in a sulphidic framework. Fungal leaching techniques have proven to be effective for metal recovery from minerals with no redox-based energy sources; these include oxides, carbonates and silicates. Fungi from the genera Aspergillus and Penicillium have been used and were seen to be quite efficient in leaching out metal values from minerals and secondary metal sources by virtue of their ability to produce secondary metabolites (organic acids, exopolysaccharides and amino acids), which facilitate effective metal chelation. Gluconic, oxalic and citric acid are the most common types of organic acids produced by fungi and are well known metal chelating agents. Fungal bioleaching involves the following mechanisms for the metal leaching activity: (i) production of organic or inorganic acids, (ii) oxidation and reduction reactions and (iii) the excretion of complexing agents. In contrast to the chemolithotrophs, fungi require organic carbon as an energy source.
The present project aims to explore the potential of fungal assisted bioleaching as an alternative or supplementary technology to conventional metallurgical processes and its potential for scale up.

Unravelling the Interaction Between Microbial Communities and the Performance of Biooxidation Tank Processes for Gold Extraction

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Keywords: BIOX®

The BIOX® technology has been applied commercially for over 30 years with the longest running BIOX® plant at Fairview mines in Barberton, South Africa. More than 12 plants are commissioned world-wide in countries such as Australia, China, Kazakhstan, Ghana, Brazil and Peru. During the development of the technology, speciation of the microbial community revealed a bacterial dominance of *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. Recent characterisation of the BIOX® microbial community revealed a significant shift from a bacterial-dominated culture to an archaeal-dominated community consisting of organisms such as *Ferroplasma acidiphilum* and *Thermoplasma sp*.

This study sets out to investigate how changes in operating conditions in the biooxidation process influence the microbial community structure and activity, such that optimal conditions for maximum gold extraction can be provided. Microorganisms from industrial samples will be enriched and/or isolated for characterisation of their physiological traits. More specifically, their mode of growth, iron and sulphur oxidation potential as well as their temperature and pH growth range will be determined. Following the characterisation of the microorganisms present in the culture, the optimal conditions and responses of the microbial community to changes in organic loading and acidity will be assessed in order to enhance process robustness.

Continuous Monitoring of BIOX® Communities: Towards Understanding Biooxidation Performance of Mixed Bacterial and Archaeal Communities

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Keywords: Bioleaching, Biooxidation, BIOX®, microbial speciation, qPCR, archaea

Microbial assisted gold leaching from sulphidic refractory ores and concentrates requires a consortium of acidophilic microorganisms comprising a balance of sulphur and iron oxidising chemotrophs. The biooxidation process is thought to be catalysed by predominantly chemolithotrophic bacterial species such as the iron oxidiser *Leptospirillum ferriphilum* and sulphur oxidiser *Acidithiobacillus caldus* with only a small proportion of other microorganisms present within these communities. As such, the microbial diversity of these biooxidation processes are seen as rather simplistic. Laboratory operated BIOX® reactors appear to retain this 'traditional' community structure. Although changes in operating conditions may result in shifts of species abundance, when operating conditions are returned to standard tank bioleaching conditions the 'traditional' community is reestablished and persists. In contrast, industrially operated reactors have been reported to be archaeal dominated (>90%) with species such as *Acidiplasma cupricumulans, Ferroplasma acidiphilum* and a *Thermoplasmatales sp* the most abundant organisms reported. Even in the absence of the 'traditional' species, this archaeal culture exhibits similar bioleaching performance under industrial plant conditions and the same pyrite/arsenopyrite mineral concentrate as used for laboratory studies. At CeBER, UCT, we have applied a qPCR toolset for the continuous monitoring of the BIOX® community associated with an industrial BIOX® operation for over four years. We have complemented these speciation events with experimental work on both the bacterial and archaeal dominated cultures to further understand the iron and sulphur oxidising potentials of these communities. In-house toolsets such as the qPCR assays used at CeBER allows a rapid community profile to be generated for specific samples. This information can aid the decision to apply changes in operating conditions to either enhance or suppress undesired characteristics elicited by the resident organisms. Supplementing the microbial community structure information with experimental work on the characterisation of mixed microbial cultures and isolates provides us with a holistic understanding of the BIOX® community. This information can be used to predict changes in the biooxidation potential and the community dynamics in response to changes in operating conditions and the characteristics of the concentrate feedstock.

The Effect of Thiocyanate on Micro-Organisms Implicated in Gold Biooxidation and its Impact on Sustainable Water Management in Biooxidation circuits

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Keywords: biooxidation, thiocyanate, inhibition, adaptation, recycling

Biooxidation, the biological pre-treatment of refractory gold concentrates, is an established technology due to its environmental sustainability and economic viability, especially for the treatment of complex concentrates. Micro-organisms associated with biooxidation operations facilitate the dissolution of the sulphide mineral matrix that encases the gold via generation of leaching agents, thereby increasing gold availability for extraction. Due to the lack of an effective alternative, cyanide remains the principal lixiviant utilised for gold extraction following biological pre-treatment. Cyanidation produces effluent waste containing various cyanide species, including thiocyanate (SCN-) and weak acid-dissociable cyanide (WAD cyanide), toxic to both the environment and the microbial community used in biological oxidation. At present mining operations are subject to increasingly stringent environmental standards and are moved to improve plant-wide water balances. This necessitates on-site effluent treatment and water recycling.

The inherent toxicity of SCN- and cyanide has prevented direct recycling of process water upstream of the biooxidation tanks where water demand is highest in associated operations. However, biological effluent treatment to degrade cyanide and SCN- has been demonstrated successfully, an example being the ASTER™ process. The ASTER™ process degrades cyanide and SCN-, producing ammonium, sulphate and cyanate in solution. The remediated wastewater may therefore be recycled within biooxidation circuits. However, systematic studies on the tolerance of biooxidation organisms to SCN- largely remains absent, thereby necessitating further research to assess the feasibility and risk of recycling remediated wastewater in the biooxidation circuit.

This project aims to determine the tolerance of keystone microbial species implicated in biooxidation operations (L*eptospirillum ferriphilum, Acidithiobacillus caldus* and *Acidiplasma cupricumulans*) to SCN-, thereby assessing the feasibility of recycling bioremediated water to biooxidation reactors. Fundamental experiments using single species as well as a mixed biooxidation consortia will provide insight into the tolerance of these organisms to SCN- as well as their potential for adaptation.

Growth Kinetics, Mineral-Microbe Interactions and Colonisation of Mixed Cultures on Whole Low-Grade Copper Sulphide Ores

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Keywords: heap bioleaching, mineral sulphides, mesophilic microorganisms, attachment

The extraction of base metals from heap bioleaching operations is often preceded an extended lag phase in microbial growth and activity during the start-up phase, resulting in poor temperature generation and progression within the heap. The present study focuses on understanding initial attachment behavior leading to the microbial colonisation of the ore, microbial growth and transport of microorganisms within the whole ore environment. Further the differing behavior across microbial species and between pure and mixed cultures will be assessed in a simulated heap leach system. Of interest is the behaviour of the most common heap leaching mesophilic microorganisms, as a better understanding of the mineral-microbe interactions of typical mesophilic microorganisms is beneficial to initial heap operation. The presence, growth and transport of pure and mixed cultures of Leptospirillum ferriphillum, Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans are studied within the different phases that exist within the whole ore system, namely, the strongly attached phase, weakly attached phase, interstitial phase and the bulk flowing Pregnant Leach Solution (PLS). In addition, the proportion of microorganisms that remain strongly attached on mineral surfaces post microbial detachment will be quantified through isothermal microcalorimetry (IMC) to assess the microbial activity of mesophilic microorganisms on the mineral surface and shed light on microbial succession.

Tomography Studies of Microscale Spatial Variations in the (Bio) Leaching of Low Grade Sulphidic Minerals

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Keywords: Heap (bio) leaching; copper minerals, X-ray microμ-CT; image processing

Major challenges in heap (bio) leaching are incomplete recoveries and long extraction times from coarse particles. The inaccessibility of non-surface mineral grains to leaching solution, which is necessary for oxidation of target mineral grains, is proposed as a key limitation resulting in these challenges. It has not been possible to study the internal structure and properties of coarse ores because traditional experimental methods involve bulk measurements, are 2D or destructive. However, X-ray μ-computed tomography (X-ray μCT) as an imaging technique allows a non-invasive and three-dimensional investigation of heap bioleaching process.

This project aims to study the effect of the operating conditions on the leaching of coarse low grade ores under unsaturated conditions at the particle and mineral grain scale using X-ray μCT, with focus on how these conditions affect sub-surface mineral leaching. Prediction of ore leaching kinetic mechanisms using X-ray μCT data beyond a bulk analysis was undertaken. Also, it has been anticipated that the association of microorganisms with the mineral surface through colonisation via generation of extracellular polymeric materials (EPS) and biofilms influences the leaching performance of bio systems over chemical systems. A premise for bioleaching studies integrating X-ray μCT is that the metabolic activity of microorganisms is not affected by X-ray energy doses. This study also evaluates the compatibility of bioleaching microorganisms with X-ray energy exposure.

The advanced 3D analysis software Avizo ® 9 was used to visualize and analyze image data to calculate reduction of sulphide minerals over the course of (bio) leaching in order to determine quantitatively iron or copper recovery and the sulphide mineral grains distance from the ore surface.

The Effect of Bed and Fluid Properties on Capillary Suction and their Impact on the Hydrodynamic Modelling of Heap Leaching Systems

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Keywords: Heap leaching, Preferential flow, Capillary suction, Hydrodynamic models

Heap leaching has carved out a niche for the treatment of low grade, precious and base metal ores due to its relatively low initial capital investment, as well as its ease of operation, foregoing the need for sophisticated heavy machinery. However, some limitations in the process has led to low recoveries, long extraction times and high operational costs at several plants around the globe. One of the main contributors to these limitations is preferential flow, defined as the uneven distribution of irrigated solution within a heap. Additionally, capillary suction forms an integral part of this non-uniformity in the spread of solution.

To better understand these phenomena and how they can be manipulated to alleviate some of the aforementioned limitations, laboratory tests are carried out to investigate the relationship between hydrodynamic parameters such as dynamic fluid velocity, diffusion, dispersion, ratio of static to dynamic liquid holdup and total saturation within an irrigated, packed bed. Some of these parameters can be obtained experimentally, however, a majority require the use of hydrodynamic models.

The aim of this study is to investigate the effect of particle shape, particle size distribution, aspect ratio, bed geometry, bed size and fluid viscosity, across typical model packing materials and ore sysems, on key hydrodynamic parameters present in five commonly used hydrodynamic models. These are the Compartmental Models (CM), Advection Dispersion Model (ADM), Piston Exchange Model (PEM), Piston Exchange-Diffusion Variant Model (PEM-D) and the Piston Dispersion with Exchange Model (PDE). The results obtained will allow for the decoupling of the effects of certain bed and fluid properties on solution flow, as well as shed light on the capabilities of these models to cope with variation in capillary effects and hence variation in preferential flow. These will form a more comprehensive foundation, upon which future modelling efforts can be constructed.

The Art of Soaking Rocks: The Systematic Study of the Transport Phenomena in Packed Rock Beds

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Keywords: heap leaching, acid rock drainage, liquid holdup, the Richards equation, hydrology

Transport phenomena play a key role in both efficiency of heap leaching operations and prevention of acid rock drainage (ARD). Despite its key role, solution flow and solute transport phenomena have been studied primarily using models. However, these models, being borrowed from the soil hydrology field, fail to connect easily measurable properties of rock beds, such as particle size distribution (PSD) and bed porosity, to their hydraulic responses. A combination of steady-state and transient gravimetric experiments, residence time distribution (RTD) studies and modelling work was conducted to provide such a link.

The results of this study have shown that there is a strong correlation between PSD and the liquid holdup. The model explaining this correlation was proposed and validated against previously reported data. In contrast, the feed flow rate, typical for heap leaching operations, was shown to have very little effect on the liquid holdup. Solute transport was demonstrated to be governed by the same physical nature across PSDs and feed flow rates. An alternative model based on RTD data and the concept of plug-flow-reactors-in-parallel was proposed.

Developing of an Integrated Process for the Sequential Extraction and Recovery of Valuable Metals from Waste Electrical and Electronic Equipment (WEEE)

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Electronic waste (e-waste) is currently the fastest growing waste in the industrialised world. With increasing demand, decreasing lifespan and rapid growth in digital technology approximately 50 million tonnes of e-waste is expected to be disposed in 2018. Waste electronic and electrical equipment (WEE) is currently discarded to landfill sites or is incinerated. However, these methods are potential health hazards with metals being leached into the environment. This results in large spread pollution and health complications in communities within 30 km of the dump epi-centre largely due to the extremely toxic nature of the PCB components.

An integral component of WEE is the printed circuit board (PCB) which consists of precious and base metals that are typically higher and more accessible than their respective virgin ores. Currently, this valuable revenue stream is being lost due to ineffective recycling techniques - the bulk of which is being recycled using crude and unregulated methods. Typically the high-value component of the generated e-waste is exported transferring beneficiation to more affluent countries. To benefit from this resource, full value recovery should occur within the economy generating it and handling the bulk e-waste load, presenting a large financial return if handled appropriately. In this project, an industrial ecology approach is taken to reduce waste burden and enhance resource recovery by investigating microbially assisted processes as a means to extract and reclaim metals associated with PCBs.

Key components of the umbrella project are presented in the four abstracts following.

Kinetic Evaluation of the Mesophilic Culture in the Presence of Waste PCBs: Inhibition and Adaptation of the Microbial Culture

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Keywords: PCBs, mesophiles, bioprocess, bioleaching, ferrous iron oxidation

Printed circuit boards (PCBs) contain various valuable metals that may be recovered and recycled. Bioleaching offers a viable process option for the recovery of base metals, subsequently allowing for improved recovery of precious metals associated with e-waste. Iron-oxidising bioleaching organisms generate the ferric iron leach agent as a metabolic by-product and thereby facilitate the solubilisation of metals embedded within e-waste material which may later be recovered. However, many of the metals associated with PCBs (copper, nickel and zinc) are known to impede activity of the bioleaching microorganisms and can therefore compromise process efficiency.

This study aims to investigate the suitability of various iron-oxidising species for application in leaching of PCBs for metal recovery. Inhibition and toxicity due to exposure to metals associated with PCBs as well as adaptation to these metals forms the primary focus of this research. This work elucidates the impact of pertinent metals on microbial activity and growth. Obtained kinetic parameters are utilised to model the microbial system and identify mode of inhibition. In addition, degree of metal toxicity is explored to define tolerable metal concentrations at which the microorganisms' activity remains uncompromised. Furthermore, the potential for microbial adaptation to metals of interest is evaluated to determine whether activity may be sustained or improved when exposed to toxic metal concentrations. This research will largely provide fundamental understanding of the microbial system and may be used to inform process operating conditions and reactor configuration.

Process Design and Optimization of Waste Electrical and Electronic Equipment

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Keywords: Modeling, reactor analysis, bioprocess, design (process simulation), printed circuit boards

The dominant formal WEEE processing route to date is pyrometallurgy, a practice that releases significant pollutants requiring extensive emission control systems to protect both the environment and surrounding communities. Additionally, these processes typically have high energy consumption rates, which contribute extensively to operating costs. In this study biohydrometallurgy is considered as a more energy efficient and less environmentally onerous process. However, this microbially assisted technology is challenged by the build-up of potentially toxic metal ions. These cations, beyond threshold concentrations, inhibit the activity of the microorganisms involved in regenerating the primary oxidant to adversely impact process performance.

To overcome these limitations, the separation of the biological turnover of ferrous iron to the primary oxidant ferric iron from the chemical leaching of the PCBs is considered. This is assessed by developing mathematical models for multiple reactor types and staged configurations. Consequence of cationic build-up is incorporated in these models and means to alleviate these are explored through inter-stage recovery and recycle. Arrangement and design of these reactor systems are evaluated by identifying the optimal trade-off between cost, energy and performance for the bioleaching of base metals from waste PCBs.

Recovery of Value From an Electronic Waste Stream Using a Biological Matrix

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Keywords: fly larvae shells, chitin, chitosan, absorption, process flowsheet

The process of metal extraction from PCBs produces a multi-metal leachate solution, which requires downstream processing to recover value. The method for selective metal recovery and value addition requires careful consideration, as the holistic approach to extracting value from e-waste is aimed at creating small to medium enterprises. Although ion exchange and solvent extraction are common technologies found in industry, absorption of metals onto biological matrices such as chitin and chitosan, is expected to adapt to scale with ease, with lower associated costs.

For this study, the source of chitin and chitosan is fly larvae, with the fly larvae shells (FLS) being a waste stream from the process using black soldier flies (BSF) to generate a protein source from waste materials. A cost-effective methodology for the extraction of chitin and chitosan from FLS is in development. Preliminary absorption studies have shown promise, achieving selective recovery of ferrous iron with chitin and selective absorption of copper with chitosan, from bimetal test solutions. The selective recovery of iron for recycling into the microbial oxidation reactor and copper as a metal sulphide from multi-metal solutions, will be achieved through integration of the absorption process with elution and precipitation methods. Metal recovery flowsheets will be proposed and assessed for techno-economic feasibility. Successful recovery of value will result in the creation of a circular economy in the waste industry, where biological waste is used to remediate electronic waste, increasing the value of both waste streams while reducing their environmental impact

Bioleaching as a Unit Operation for the Recovery of Copper and Other Metal of Value from WEEE.

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Keywords: Printed circuit boards (PBCs), chemical kinetics, microbial immobilisation

The results from preliminary studies conducted at CeBER have suggested that enhanced extraction of valuable metals from PCBs may be achieved with the separation of the two sub-processes involved, i.e. the chemical leaching of PCBs from microbial ferrous iron oxidation, into two reactor systems. This two-stage system is expected to minimise microbial inhibition due to metal toxicity and exposure to PCB resin, thereby facilitating the growth and activity of a more robust microbial population while allowing tailoring of conditions for each sub-process.

In this study, the chemical leach kinetics of Cu, Ni, Zn, Sn and Fe metals, together with microbial oxidation kinetics in the absence of inhibition, are quantified as a precursor to informing the design of the two-stage reactor system. This will allow the tailoring of the integrated process to match associated sub-process rates. Further it will deliver appropriate kinetic data and resultant rate equations to inform two-stage process design. A significant part of the study focuses on determining the optimum operating conditions required to achieve maximum specific microbial oxidation rates for a given reactor configuration. This aspect of the study will include microbial adaptation and immobilisation as techniques to counter potential microbial inhibition and enhance ferric iron regeneration. Data gathered will also inform the operation of the integrated process for the extraction of valuable metals from PCBs.

BIOPRODUCTS

The Pulp and Paper Wastewater Biorefinery: Potential for Concomitant Value Recovery and Wastewater Treatment

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Keywords: Bioproducts, valorisation; fit-for-purpose

The Pulp and Paper Wastewater Biorefinery (P&P WWBR) project is an extension of the WWBR concept introduced in the K5/WRC 2380 report. The previous report recognises the potential of wastewater (WW) as a valuable resource and proposes the combination of biorefining operations and wastewater treatment to produce both clean, 'fit-for-purpose' water and value added bioproducts. P&P WW is usually complex and high volume in nature, making it suitable for a WWBR. Over its 4 years' time span, our project will deliver literature reviews and case studies to inform on the possible implementation of a P&P WWBR in South Africa. Through our literature review, we are able to identify the lignocellulosic potential of the P&P WW and identify the degree of pollution in different WW streams in a P&P facility. We are further reviewing various ways which global P&P facilities employ to valorise their waste streams and assessing their solutions within the South African context. A major challenge lies in the identification of suitable bioproducts to ensure the economic viability of the P&P WWBR. A preliminary product selection guide has been put together to streamline the various products with regards to market compatibility and their sustainable production from WW. Our case studies are site-specific and involve the postgraduate work of CeBER students who will develop integrated process flowsheets along with techno-economics and laboratory work pertaining to products synthesis from P&P WW. As part of the implementation process, we are also mapping the various P&P mills in South Africa and grouping them in regional clusters in order to propose possible collaboration among mills who wish to valorise their wastewater. Based on our findings and results, we can propose an implementation framework with regards to valorising WW along with the adequate bioproducts selection, which can be transferable to other sectors.

Reference:
Harrison STL; Verster B; Rumjeet S; Raper T; Rademeyer S; Johnstone-Robertson M; Mosters L, 2016. *Towards Wastewater Biorefineries: integrated bioreactor*

The Use of Pulp and Paper Wastewater as a Potential Feedstock for Lignocellulosic Enzyme Production

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Keywords: Biorefineries, circular economy, bioproduct; effluent

The concept of wastewater biorefineries aims to move the industrial production towards reusing generated wastewaters components of value while treating wastes and recovering clean water as a product, which can contribute to creating circular economy in line the concepts of industrial ecology and cleaner production. Pulp and paper industry is one of the major contributors in generating wastewater, the waste generated in pulp and paper ends up in landfills sites, piled up and sometimes discarded in aquatic environments. This project focuses on the new biological approach towards wastewater treatment by utilising pulp and paper wastewater effluents as a potential feedstock for bioproduct mainly enzyme production. The aim is to select potential lignocellulose-degrading enzymes as a product of interest based on production efficiency, cost production, yields, industrial competitiveness and demand in the pulp and paper sector. These enzymes are used as a biotechnological tool and find application in the pulp and paper industry, either to pre-treat the feedstocks for the (wastewater biorefinery) or to pre-treat the feedstocks to facilitate wastewater treatment. Pulp and paper sludges potentially offer a niche environment to the bacteria and fungi producing these enzymes, the focus being fungi. Selected fungal species with high levels of efficient lignocellulosic degradation Trametes versicolor, Trametes pubescens, Phanerochaete chrysosperium and Aspergillus niger. Selected appropriate streams will be considered from specific mills and characterised.

The Wastewater Biorefinery: Improving the Sustainability of the Pulp and Paper Industry

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Keywords: Wastewater biorefinery, pulp and paper, Resource efficiency, bioremediation, waste valorization; biocommodities

Pulp and paper mills consume large amounts of water. Considering South Africa's limited water supply and an increasing focus on resource efficiency and the circular economy, efficiencies in water use and re-use are sought. The wastewater streams discharged from the pulp and paper plants have a high chemical oxygen demand (COD), indicative of inorganic and organic compounds being present in the wastewater. The required reduction in fresh water intake can be met only in part by improving operations and running the mill more efficiently. The Wastewater Biorefinery (WWBR) is well-suited to extending this solution. The WWBR refers to a biorefinery consisting of multiple units that runs in parallel with an existing production plant, using the wastewater from the existing plant as feedstock for the production of biocommodities and bioenergy. The aim of a WWBR is to maximise resource productivity whilst lowering environmental impact and optimising further use or re-use of the water stream, without increasing economic burden.

The primary biocommodity chosen for production from pulp and paper wastewater is lactic acid, a platform chemical with a broad range of applications including the production of poly lactic acid (PLA), a biocompatible, biodegradable plastic. It is proposed that lactic acid is produced from the primary sludge from the wastewater treatment process, a lignocellulose-rich stream. This requires the lignocellulose to be broken down into glucose and xylose monomers by enzymes followed by the anaerobic fermentation of these monomers to lactic acid by thermotolerant *Bacillus coagulans DSM 2314*, and subsequent recovery.

The project considers the development of the integrated WWBR around the central lactic acid train. The focus for the additional units is on achieving 'fit-fr-purpose' water quality by removing the remaining components. Currently, the flowsheet contains an anaerobic digestion unit to remove residual organics, a macrophyte reactor for polishing the water and a fungal reactor for the production of enzymes. The final flowsheet will provide insight to the pulp and paper industry on approaches to overcome water consumption limitations and improve resource efficiency in a climate where the biocommodities are gaining momentum and the implementation of green technology is being more actively pursued.

Investigating Red Pigment Production by *Penicillium Purpurogenum* DSM 62866

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Keywords: Natural colourants, filamentous fungi, medium composition

Currently, there is a move towards the use of natural colourant alternatives in food, cosmetics, nutraceuticals and other related products. This results from consumer preference for products which are perceived to be natural and healthy, the negative environmental and health implications of synthetic colourants and government legislation limiting the application of certain artificial colourants previously used. The majority of natural colourants in use are obtained from plant and insect sources and are affected by limitations including natural variation, low yield of product from the source and seasonal availability.

Microorganisms have garnered interest as potential sources of alternative natural colourants. They can produce pigments of similar colour and chemical structure, while overcoming some of the limitations of other natural pigment sources. Filamentous fungi produce a diverse range of pigments and therefore represent an interesting alternative source of natural colourants. Fungi additionally have great potential for large-scale cultivation under controlled conditions, with minimal dependence on weather and seasonal changes. The filamentous fungus *Penicillium purpurogenum* was selected for investigation in this study on the basis of reported pigment type and colour produced, the absence of mycotoxin production and the ability to alter pigmentation through modifying cultivation conditions. The red pigments produced by this fungus are of particular interest as a result of commercial demand for pigments in this colour range.

This study aims to investigate pigment production by the organism *P. purpurogenum DSM 62866*. This will include determining the growth and pigment production response to changes in cultivation conditions and medium composition, investigating the potential for using a confectionery waste stream as a nutrient source, cultivation scale-up and characterisation of the pigmentation produced. This work will address the current gap which exists in curiosity- and discovery-based studies, which currently dominate the microbial pigment production space, by investigating the factors affecting process yields and productivity, aiding the move towards a robust process for pigment production. This study will provide insight into whether potential exists for commercial production and application of natural red colourant alternatives using *P. purpurogenum DSM 62866*.

Evaluation of The Impact of Bioreactor Type on Gas/Liquid Mass Transfer and Productivity in Dense Aerobic Yeast Culture

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Keywords: kLa, oxygen, yeast, mass transfer, fed-batch

Owing to the low profit margin associated with yeast production, product yield is of high importance. High yields are maintained by utilizing an aerated fed-batch process, preventing the Crabtree effect from occurring and ensuring that the ethanol production pathway is avoided. With an appropriate feeding strategy and adequate heating/cooling systems in place, the limiting factor in yeast cell production is oxygen transfer as the increasing biomass density requires increasing amounts of oxygen. Hence, bioreactors which can provide enough oxygen transfer capability are preferred in the industrial production of yeast. Conventional bioreactors employed in the bioprocess industry include stirred tank, airlift and bubble column reactors. Bioreactor selection depends on the maximum oxygen transfer obtainable by the bioreactor, which in turn is dependent largely on the gas-liquid mass transfer coefficient (kLa). This subsequently contributes to the power efficiency through which a sufficiently high kLa can be obtained to meet a target productivity and final biomass concentration. Information on the range of kLa values obtainable by each bioreactor system and the associated energy efficiency in power input per unit volume is limited in literature, especially for more niche bioreactors such as the wave bioreactor. In this work, this kLa range and power efficiency for stirred tank reactors (established technology) and wave bioreactors (emerging technology) are investigated. The kLa vs power input per unit volume of both reactor systems is compared, as well as the productivity and yield of a yeast fermentation performed in both systems.

Poly (γ-glutamic) Acid (PGA) Production From Confectionery Waste

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Keywords: biorefinery, renewable resource, *Bacillus*, resource recovery

South Africa's confectionery industry contributes to a significant proportion of the country's economy. The treatment of confectionery waste, is costly because of the high chemical oxygen demand (COD); as a result much of this waste is sent to landfill, with associated negative environmental impacts. The South African Department of Science and Technology Waste Research, Development and Innovation roadmap initiative (Waste RDI) is to minimise waste entering landfills by identifying waste sources from which to produce value to contribute to social and economic growth. Confectionery waste has a high sugar content which can be used for feedstock to bioprocesses. By placing this bioproduction into a waste biorefinery framework, bio-based raw materials can be used to produce competitively priced products with low environmental impact, thereby optimising remediation and value generation simultaneously. Polyglutamic acid (PGA) was selected as a potential product from confectionary waste as it is a high value product that has many applications. This study explored the use of hard candy waste as a potential feedstock for PGA production using *Bacillus licheniformis* JCM 2505 in 7 L stirred tank reactor(s). Results showed that PGA is a growth-associated product, provided that there was adequate sucrose and nitrogen within the system. To increase the productivity and yield of PGA, duplicate fed-batch experiments were run at the constant feeding of candy and NH_4Cl solution at 0.0833 L/h in the C:N ratio needed for optimal PGA production. The biomass concentration was increased from 5.86±0.68 g/L to 10.3±0.81 g/L, while the PGA productivity remained unchanged.

Polyhydroxyalkanoates Production From Confectionery Waste

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Keywords: Biopolymer, value-added product; bioproduct

The solid waste generated by the confectionery industry is rich in carbohydrates, fat and proteins which are the main constituents of confectionery products. The South Africa confectionary industry is one of the well-established food industries in Africa. Each year, it generates substantial amounts of confectionery waste, rich in easily digestable organic compounds which are discarded into land fill. As part of waste management strategies, South Africa has introduced a directive aiming to divert waste from landfills toward value addition through the Research Development and Innovation Roadmap 2014. Polyhydroxyalkanoates (PHAs) have been identified as one of the potential value-add products that can be obtained from carbohydrate rich waste substrates such as confectionary waste. Polyhydroxyalkanoates are polyesters produced from renewable resources microbially, as a storage product, through bioprocess technologies. Their application as bioplastics make them suitable as a potential alternative to conventional plastic due physical, mechanical and eco-friendly properties such as biodegradability and biocompatibility. Mostly, they are considered for niche applications associated with uses requiring biogradability,for example in healthcare products, agriculture, medicine and pharmacy. However, the high cost associated with their production is still restricting their expansion. This has been largely attributed to the cost of the raw material for their production. This study aims at using the confectionary waste as the alternative substrate for the production of polyhydroxyalkanoates using *Alcaligenes latus* and *Cupriavidus Necator*, two well PHA producers. This provides potential for integrating a cheaper raw material for their production as well as finding an alternative use for confectionary waste toward value addition, thereby enhancing resource productivity. The results on the yield and productivities will guide a techno economic analysis and potential biorefinery integration.

A Feasibility Study of Confectionery Waste as a Substrate for Renewable Energy Production

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Keywords: bioenergy; waste valorization; bioproducts

Annually, the confectionery industry generates approx. 625 tonnes of sugar-rich waste per manufacturing site which is discarded into landfills, presenting a major environmental burden owing to its uncontrolled bioconversion to organic acids which facilitate leachate generation and of methane and CO_2, both greenhouse gases. The carbohydrate content of confectionery waste makes it an ideal bioprocess feedstock for renewable energy production. Valorisation of confectionery waste contributes to the national Bio-economy strategy (2014) of SA that focuses on diverting waste from landfill sites into value added products using sustainable and environmentally friendly production processes. This project is aimed at investigating the feasibility of confectionery waste as a suitable feedstock for renewable energy production. Three approaches to bioenergy production will be investigated: 1) Bioethanol production using *Zymomonas mobilis*; 2) Biobutanol production by *Clostridium saccharobutylicum* and 3) Biogas rich in either methane (CH_4) or hydrogen (H_2) generated by a consortium of anaerobic microorganisms.

Research to date has been focused on bioethanol production using *Z. mobilis* ATCC 31821. Z. mobilis was cultivated anaerobically in medium containing confectionery or synthetic sugar mixes to simulate products such as hard candy, chocolate or marshmallows as carbon (C) source supplemented with essential nutrients. Ethanol yields obtained from the confectionery waste streams were lower than those obtained in defined media. This was attributed to unknown additives in the confectionery products. The study now focuses on improving ethanol yields from confectionery products to that comparable to defined media.

Energy generation from waste streams is a sustainable approach for energy recycling during confectionery manufacturing. These bioenergy sources can be used to supplement the processing plant's energy requirements. Biofuels and biogas can be used directly in heating boilers to generate steam to supply electrically powered steam generators located alongside processing equipment. Therefore, this study aims to provide an overall comparison of the proposed energy routes using experimental data and techno-economic analyses of the process technologies and products.

Novel Processes for Efficient Bioethanol Fermentation From the Mixture of Glucose and Xylose using *Zymomonas Mobilis* and *Pichia Stipitis*

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Keywords: Reactors in series, sequential batch culture; bioproducts; bioethanol

High and efficient conversion of glucose and xylose is necessary for the commercial viability of bioethanol production. In this research, fermentation process conditions were optimized (e.g. pH, temperature, process configuration) and implemented (e.g. immobilization of cells, high inoculum sizes and fluidized bed) to increase ethanol yields and productivities from the mixture of glucose and xylose using *Zymomonas mobilis* and *Pichia stipitis*. Co-culture is an option for bioethanol fermentation from a mixture of glucose and xylose as currently there are no high ethanol production wild type strains capable of utilizing both sugars efficiently. However, issues relating to slow xylose utilization, low ethanol tolerance of the xylose-fermenting cells and different optimum process conditions for different microorganisms remains. In order to solve these concerns, two-stage sequential batch fermentations in one reactor – first stage for glucose-fermenting microorganisms (suspended or immobilized *Z. mobilis*) followed by xylose-fermenting organisms (suspended or immobilized *P. stipitis*) to produce ethanol were tested. In the susoended cells setup the volumetric ethanol productivity of 0.73 g L^{-1} h^{-1} and an ethanol yield of 0.47 g g^{-1} were obtained. When the cells were immobilized, the productivity dropped to 0.44 g L^{-1} h^{-1} and the ethanol yield to 0.45 g g^{-1}. Alternatively, continuous two-stage fermentations in two reactors in series were tested to increase bioethanol yield and productivity from the mixture of glucose and xylose using *Z. mobilis* and *P. stipitis*. The continuous culture in two reactors in series with immobilized *Z. mobilis* in a stirred tank reactor with low agitation rate of 50 rpm for glucose consumption; and suspended *P. stipitis* in a stirred tank reactor with agitation rate of 200 rpm in the second forxylose fermentation did not result in significantly higher ethanol productivity (0.713 g L^{-1} h^{-1}) and yield (0.43 g g^{-1}). When the continuous culture were configured to immobilized *Z. mobilis* in a nitrogen sparged fluidized bed reactor (glucose consumption); and suspended *P. stipitis* in a stirred tank reactor with agitation rate of 200 rpm (xylose fermentation), higher ethanol yield and productivity (0.48 g g^{-1} and 1.14 g L^{-1} h^{-1}) were achieved.

Process Technologies for the Recovery of Biobutanol via *in-situ* and *ex-situ* Separation

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Keywords: Biobutanol, sugarcane, process modelling, fermentation; bioproducts

Butanol is an important chemical feedstock, derived synthetically from both coal and crude oil and a biological fermentation route, with the former currently the dominant route. It has a plethora of uses in the chemical and manufacturing industries. Biobutanol (mostly *n*-butanol but including *iso*butanol) has also been accepted as the preferred biofuel over bioethanol due to its favourable properties of low hygroscopy and higher energy density. This makes it suitable as a "drop-in" bio-based chemical to the existing petrochemical infrastructure. In South Africa, biobutanol is being investigated as a potential platform chemical, as part of the initiative from the Sugar Milling Research Institute (SMRI) to add value to the declining sugarcane industry through the STEP-BIO Biorefinery Technoeconomic Model (BRTEM) project. The bio-based production of butanol, however, faces the challenges of low yields from starchy or sugar-based feedstocks, low productivities (slow fermentation) and an energy intensive separation. This project focuses on the separation and recovery of biobutanol from the fermentation culture via integrated separation (*in-situ* and *ex-situ*) towards improving the energy efficiency and reducing product inhibition to enhance productivity. Biological kinetic studies will be undertaken using *Clostridium saccharobutylicum* P262 in batch and continuous fermentations with integrated separation to determine the effects of the separation on the biocatalyst, yield and productivity. Using these results, rigorous process simulations will be developed to assess the technical feasibility of proposed process technologies and determine the overall energy benefit as compared to the traditional purification via distillation.

Bioremediation of vinasse with associated value recovery through its pretreatment and anaerobic digestion

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Keywords: Remediation, bioproducts; sugarcane production; bioethanol

The increase in demand and production of bioethanol from sugarcane molasses has intensified the search for reliable and sustainable means of treating the wastewater produced. This wastewater, commonly called vinasse, is produced in large volumes (25 L of vinasse per 1 L of ethanol) and has been characterised to have a high chemical oxygen demand, which is attributed to the presence of salts, caramels, melanoids, unfermented sugars and phenolic compounds. The complexity of vinasse, coupled with its acidic pH and dark colouring prevents challenge for vinasse remediation. Due to the high volume and the complex nature of the vinasse, failure to adequately treat vinasse (at a low cost) will place constraints on bioethanol production. New approaches are sought to handle the increasing vinasse volumes.

Anaerobic digestion (AD) is recognised as a potential and robust technology to remediate organic waste streams whilst producing new clean fuels, either in the form of hydrogen or methane. As vinasse is largely a liquid organic waste stream, anaerobic digestion for organic removal and methane generation is a promising approach to value addition of vinasse. However, studies to date on the anaerobic digestion of vinasse have been shown numerus obstacles. In particular, inhibition of the AD process due to the presence of interfering compounds occurs. This study investigates the key inhibition compounds, pretreatment of vinasse for their removal and the potential for value generation and remediation of sugar cane molasses vinasse produced in South Africa. The study focuses on pretreatment of vinasse in the form of desalting to improve AD performance and increase the possible products generated from vinasse remediation. It also addresses the impact of these inhibitors on the AD process.

The role of rhizodeposition and microbial communities in plant microbial fuel cells

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Keywords: Bioelectricity; exoelectrogenic microorganisms; electrical power

The plant microbial fuel cell (PMFC) is an emerging technology that utilizes plant rhizodeposition and extracellular electron transfer by exoelectrogenic microorganisms to generate electricity. This technology does not generate combustion-based emissions and it can be integrated into decentralized electricity generation plans in the future. Plants transfer some 20-40% of photosynthetically produced organic compounds into the soil by rhizodeposition. The rhizodeposits typically contain sugars, organic acids, enzymes and dead cell material that can be oxidised by exoelectrogenic microorganisms. The electrons generated by these microorganisms during oxidation can be transported externally through a circuit to generate electrical power. Whilst PMFC studies have postulated correlations between the presence of plants and power generation, there are very few studies that demonstrate the direct effect of rhizodeposits on power output. This investigation attempt to characterise plant rhizodeposit composition, its interaction with microbial communities in plant growth media and its corresponding effect on power generation. The study considers the growth of tomato plants in hydroponic and unsaturated plant growth media in a controlled growth environment. Rhizodeposits of the tomato plant and their influence on the natural microbial communities and the exoelectrogen *(Shewanella putrefaciens)* will be analysed closely with the power output of the system. Key answers from this study may be used to test a pilot-scale PMFC system for electricity generation in South Africa.

Mechanisms and Enzymes in the Biodegradation of Plastics

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Keywords: Plastic waste, biodegradation, polyethylene, polystyrene, thermostable enzymes, bioprocess

Over a million tons of plastic wastes are disposed of in landfill in South Africa every year, due to the under-development of the recycling industry and a general lack of innovative waste conversion technology. This includes large amounts of polyethylene (PE), as well as polystyrene (PS), which represents a neglected waste stream in South Africa. This type of plastic, among others, is difficult to recycle mechanically and thus provides opportunities for alternative waste beneficiation strategies. Biological valorization represents a potential means for the management of non-recyclable plastic waste in line with circular economy objectives. The bioconversion of plastic materials by microorganisms to yield higher value fine and speciality chemical products has been reported in literature but requires optimization and engineering to improve economic viability.

The study seeks to address gaps in the literature regarding the effectiveness of different enzyme types involved in degradation of PS and PE, as well as the contribution of factors limiting bioavailability and reaction rates. Key enzymes have been identified from literature studies and knowledge will be augmented by identification of contributing enzymes from the plastic-degrading isolate *Sporobolomyces roseus* secured and identified in CeBER. Molecular mobility has been proposed as one of the main determining factors of reaction rates in the enzymatic degradation of polymers, and this hypothesis will be tested by utilizing the hyperthermostable laccase enzyme from *Thermus thermophilus* in high temperature degradation experiments using different substrates. Additionally, the influence on reaction rates of particle size, chemical identity and molecular weight of various polymer compounds will be investigated. The study is expected to work towards the underpinning of the future development of biologically mediated plastic waste conversion processes, ultimately representing substantial environmental and commercial benefits.

Identifying and mitigating key limitations in the biotransformation of n-octane to 1-octanol by whole-cell Escherichia coli expressing CYP153A6

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Keywords: CYP153A6, paraffin activation, 1-octanol, biotransformation bottlenecks

The functionalisation of saturated hydrocarbons to create platform chemicals provides opportunity for significant value addition. However, established processes for the activation of alkanes operate under extreme conditions and suffer from low selectivity. Enzymatic oxyfunctionalisation offers a mild and highly selective alternative. Unfortunately, purified enzyme systems demonstrate extremely low stability, particularly in hydrocarbon-based systems. While whole-cell biocatalysts allow for extended run times, rates achieved thus far are well below the demonstrated kinetic capabilities of CYP153A6.[1–4]

The biocatalytic process involves numerous interacting factors and could therefore be limited in a variety of ways. This study considered four factors that can be easily controlled through genetic or process engineering: intracellular concentration of the CYP153A6 enzyme, which was varied through the addition of different concentrations of haem precursors to the growth medium; oxygen supply, which was varied by changing the volume of the vessel headspace, or by replacing the air in the headspace at regular intervals; regeneration of the NADH cofactor that supplies the electrons necessary for biotransformation, which was varied by the co-expression of a heterologous glycerol dehydrogenase; and transport of octane and 1-octanol across the cell membrane, which was enhanced through physically breaking the membrane, addition of chemical permeabilisers, or expression of the hydrocarbon-specific channel protein AlkL.

Production of 1-octanol was found to be independent of intracellular CYP153A6 concentration; volumetric performance depended on overall biocatalyst concentration (cell density). Oxygen concentration was not limiting in low-density cultures; it may have become limiting in high-density cultures, but only at later time points. Enhanced cofactor regeneration gave mixed results, interacting strongly with other physiological factors. Cross-membrane transport was shown to play a significant role in biotransformation: chemical permeabilisation improved initial rates but compromised stability; partial mechanical breakage was found to be a more effective approach over longer periods. AlkL expression also improved performance, although the relative benefits diminished as the biotransformation progressed.

Whole-Cell Hydroxylation of Longer Chain Alkanes via CYP153 Enzymes

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Keywords: Biocatalysis, higher-value fine chemicals, CYP153A enzyme, alkane activation, regio- and chemo selectivity, liquid biphasic bioprocess

Linear alkanes are abundantly available, inexpensive and produced from expanding activities in the gas-to-liquid (GTL) fuel industry. There is an international interest in the utilization of this material not only as low value fuels and solvents, but also as a feedstock to produce higher-value fine chemicals. Alkane functionalization via chemical catalysis is limited by low selectivity at elevated thermal conditions. These extreme environments increase the likelihood of over-oxidation of valuable products, which may be an economic burden due to multiple downstream purification requirements. Biocatalysis has shown outstanding regio- and chemo-selectivity for the oxidation of alkanes at near ambient conditions. The CYP153A family are P450 monooxygenases are uniquely capable of terminal carbon hydroxylation with pronounced regioselectivities relative to other biocatalytic enzymes or chemical catalysts. However, economic and industrial implementation of biocatalysis is limited by stability, cost, low product titres, waste generation and downstream processing complications. Whole-cell biocatalysis may provide a solution. These systems are cheaper than the use of cell-free enzymes as the cells are self-sufficient, protective and regenerate expensive cofactors as needed. As activation reactions usually involve hydrophobic substrates, unconventional non-aqueous biocatalyst media and process systems are necessary. The biphasic water-immiscible organic-solvent system is widely known solution. In this study, biphasic systems are explored as a means to overcome common process limitations such as substrate availability, delivery and access to the biocatalyst in the bio-functionalization of long chain alkane (C_8 – C_{12}) that ultimately constrain bio-catalytic activity. These inhibitions arise from poor mass transfer and thermodynamic limitations, low substrate solubility and therefore uptake into the whole-cell membrane.

Optimizing the Physiological Conditions for CYP153A6 Mediated n-octane Activation, Within a Growing *E.coli* Biphasic Bioprocess

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Keywords: Alkane activation, n-octane, 1-octanol, *E. coli*, growing-system, bioprocess, optimization

Naphtha is a mixture of hydrocarbons with 6-12 carbons which is derived from the fractional distillation of crude oil. Naphtha can be classified into two main categories, light naphtha which consists of light hydrocarbons with 6 or less carbons and heavy naphtha consisting of hydrocarbons exceeding 6 carbons. Heavy naphtha is either used as an additive to petrol or is catalytically reformed to produce high-octane petroleum components. Light Naphtha currently has low industrial value compared to heavy naphtha. Catalysis could provide a pathway to better utilize low valued hydrocarbons and, through conversion, higher valued product(s) generated with greater industrial application.

One way of adding value to hydrocarbon resources such as that of light naphtha, is by selectively adding a reactive group to the terminal carbons of linear alkanes (n-alkanes). Such alkane activation is the addition of an alcohol group (R-OH) to the terminal carbon(s) resulting in a product with increased reactivity and therefore value. The addition of reactive groups to alkanes adds value as these hydrocarbon pools can become feedstock for currently established industries and therefore is of great interest.

Hydroxylation of n-alkanes via synthetic catalysis has proven to be challenging and of little benefit to industry due to the spectrum of by-products generated. In contrast, alkane activation by biocatalysis yields specifically targeted product(s) streams with greatly reduced byproduct formation reducing downstream purification costs. One such biocatalyst is CYP153A6, a cytochrome p450 enzyme from *Mycobacterium sp. HXN-1500.* CYP153A6 can hydroxylate short to medium chain alkanes with the highest affinity exhibited towards n-octane. This research project aims to identify optimal process conditions for CYP153A6 mediated n-octane activation within a growing *E. coli* cell system, with particular focus on operating pH and temperature.

Cell-free bio-conversion of octane to octanol via a bio-catalytic approach

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Keywords: Immobilization, cytochrome P450, octane, biotransformation

Linear alkanes are produced as by-products from the increasing use of gas-to-liquid fuel (GTL) technologies and can be activated to produce useful chemicals such as alcohols, aldehydes, carboxylic acids and other derivatives. Activation of these unsaturated aliphatic chains is challenging due to the inert nature of the hydrocarbon backbone. Cytochrome P450 monooxygenases (CYPs) are enzymes that can be used for activation of these aliphatic alkanes using whole cells or cell free systems. P450 systems are rarely investigated as cell-free systems. Consequently, the study explores the immobilization of a self-sufficient fused CYP102A1 87-328NADH mutant and glucose dehydrogenase (GDH) via their His-tag. This approach potentially offers increased stability, reusability and easy recovery of the immobilized biocatalyst. Presently, the CYP102A1 mutant and GDH (for co-factor regeneration) have been expressed in *E. coli* and purified by metal affinity chromatography. Various immobilization methods have also been investigated including surface attachment on MagReSyn® microspheres with various functionalities and metal functionalized iminodiacetic acid (IDA) agarose beads as well as self-assembly based methods forming Cross Linked Enzymes (CLE), Cross Linked Enzyme Aggregates (CLEAs) and spherezymes. Initial experiments with GDH have shown the most potential when immobilized via their His-tag. This immobilization strategy was therefore also used to successfully immobilize (and simultaneously purify) the CYP102A1 mutant. Characterization and comparison with the free enzymes in terms of storage stability as well as their reusability will also be considered. It is anticipated that the proposed immobilization system will offer enhanced enzyme stability, reusability and easy recovery with continuous co-factor regeneration. These features provide a positive outlook on this robust multi-enzyme system for efficient activation of linear alkanes as well as the potential for immobilization of various multiple enzymes, including multimeric enzymes for different bio-catalytic applications beyond alkane activation.

Tandem Bio-Chemo Catalytic Systems for Further Alkane Oxidation to Aldehydes Using a Biofabricated Pd-Au Catalyst on *Escherichia Coli*

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Keywords: Green chemistry, bimetallic catalyst, biofabrication, alkane activation

Within the chemical industry recent interest has been focused on the activation of alkanes generated from the petrochemical sector. Alkanes are extremely stable compounds; they have the potential to become low-value feedstock for the conversion to higher value chemicals (Bergman 2007; Labinger & Bercaw 2002). This study will incorporate the use of a tandem bio-chemo catalytic processes to ensure the conversion of alkanes to alcohols and subsequently to higher value aldehydes. The chemo-catalytic oxidation of the alcohol to the aldehyde will be the primary focus of this study.

The chemo-catalytic approach considers the use of metal nanoparticles; this catalyst structure exhibits positive attributes of both homogeneous and heterogeneous catalysis (Bennett et al. 2013). Typical processes for metal recovery and the generation of metal nanocatalysts on synthetic supports make use of harsh chemicals and can be expensive to carry out. However, certain microorganisms have a natural affinity for the adsorption of metals. This is considered to be a result of the hydrogenase enzyme (Lloyd et al. 1998; Mikheenko et al. 2008). Various bacteria have been studied as scaffolds; *Escherichia coli*, a facultative anaerobe, has exhibited the ability to produce uniform, monodispersed metal nanoparticles. Furthermore, this support was shown to have catalytic activity comparable to standard synthetic alternatives. In particular, the biofabricated Pd-Au catalyst has shown near complete selectivity for the oxidation of alcohols to aldehydes (Deplanche et al, 2011)

In accordance with 'Green chemistry' practices, the biofabricated catalyst is selected as a benign alternative to standard synthetic supports. Furthermore, it has the potential to be applied as a technique for remediation of metals from contaminated water systems and recovery of precious metals from secondary waste streams (Macaskie et al, 2017).

Development of Novel Cell Culture Bioreactor and Systems for Biopharmaceutical Products

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Keywords: Horizontal tubular bioreactor, spiral impeller, shear sensitive, suspension culture, Integrins, Isothermal microcalorimetry

Mammalian cells offer superior cellular machinery for the production of complex biological products. These cells provide proper post-translational processing machinery for recombinant protein expression for the desired folding for optimal activity. However, mammalian cells are shear sensitive have low growth rates and cell densities. The continuous increasing demand for complex products has therefore driven the development of new bioreactor designs and processes to tackle these issues. Existing bioreactor designs exert high shear forces due to impeller speed, bubble disruption, foam formation and improper mass transfer at a large scale, impairing the performance of cell lines. Achieving high cell density and prolonged viability with correct glycosylation of secreted protein is still a challenge during scale-up of these designs. For adherent cells, use of microcarriers elevates the cost of production and scaling-up of these systems are costly and technically challenging. In this research, two approaches are investigated to address these challenges. Firstly, a horizontal tubular bioreactor (HTB) with a spiral impeller was designed and fabricated for the propagation of mammalian cells with a focus to achieve high cell density by improving mass transfer whilst reducing hydrodynamic shear and energy requirements through surface aeration. A value of kLa (16 h^{-1}) and cell density of 4.2 x 10^6 cells.mL^{-1} were achieved in a single batch. Secondly, the adaptation of adherent Chinese Hamster Ovary (CHO) and Vero cells into suspension culture with serum-free media was investigated. The absence of a supporting surface for cell growth and serum-free conditions are expected to reduce the cost of manufacturing and to achieve a higher productivity of protein production. CHO cells have been successfully adapted to suspension in serum-free conditions and propagated in HTB whereas Vero cells have been adapted successfully to serum-free media in adherent conditions. To adapt the Vero cells into suspension, an anti-cancerous drug is used to suppress the expression of integrin which is a cell adhesion receptor. Isothermal microcalorimetry is then be used to evaluate the viability and vitality of the Vero cells before and after drug treatment. The outcome of this research would open a new window to adapt cell-lines into suspension in a single step.

Scale-up and Optimisation of Lactobacilli Probiotic Production for the Treatment of Bacterial Vaginosis

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Keywords: Probiotics, Lactobacillus, Bacterial Vaginosis, Scale-up, Microbiota.

Bacterial vaginosis is a prevalent disease which occurs in women of child-bearing age. Bacterial vaginosis is characterised by a depletion of commensal *Lactobacillus* species in the female lower reproductive tract (LRT) and, thus, an overgrowth of pathogenic microbes including *Gardnerella vaginalis*, genital mycoplasmas, and anaerobic Gram-negative rods and Gram-positive cocci (Beigi *et al.*, 2004). This shift in the female LRT microbiome is known to have adverse effects such as the woman being 3.2 times more prone to contracting HIV and STIs, premature labour and spontaneous abortions. Furthermore, the female LRT microbiome is said to be affected by ethnicity and geography (Happel *et al.*, 2017). South Africa does not have sufficient probiotic products tailored for the vaginal health of the endemic population (Happel *et al.*, 2017).

Bacterial vaginosis is treated using antibiotics such as clindamycin and metronidazole. The antibiotics have similar clinical efficacy; however, the pathogenic microbes build resistance against the antibiotics and there is a 6-month 50% post-treatment recurrence rate following antibiotic treatment (Happel *et al.*, 2017). This serves as motivation for alternative means of addressing the issue of bacterial vaginosis. This research project aims to explore the application of live biotherapeutics, in the form of probiotics, to treat bacterial vaginosis. Probiotics are defined as live bacteria or yeasts that when taken in adequate amounts, confer health benefits to the host.

Four strains of *Lactobacillus* species have been identified as potential candidates for application in probiotics. *Lactobacilli* produce lactic acid and hydrogen-peroxide as bactericidal and virucidal compounds against pathogenic microbes. These two compounds, primarily lactic acid, reduce the pH of the vaginal milieu and makes habitation unfavourable for pathogens. The lactic acid produced by the *Lactobacilli* comes in two isomers and each isomer of lactic acid serve a different role in protecting the vagina. Additionally, the *Lactobacilli* species outcompete pathogenic microbes on the surface of the vaginal epithelial cells. These key characteristics were used in selecting the identified *Lactobacilli* best suited for this application as probiotics.

The further aim of this research project is to scale-up and optimise the production of *Lactobacilli* probiotics. Literature suggests that that the usage of a multi-strain probiotic consortium is expected to have a better efficacy than a mono-strain probiotics. Thus, the production of *Lactobacillus*, both in pure culture and co-culture, is intended to be scaled up from 100 ml shake-flasks to a 70 L bioreactor.

Process Optimisation and Scale-Up for the Production of H22(scFv)-ETA' Targeting CD64 in Acute Myeloid Leukemia (AML)

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Key words: Immunotherapy, H22(scFv)-ETA'; AML; osmotic stress; process optimisation, KLa, scale-up

Immunotherapy presents a paradigm shift as a latest knowledge-based innovative cancer treatment modality. H22(scFv)-ETA' is an immunotherapeutic recombinant fusion protein which has been shown to be highly potent in selectively destroying CD64-positive M1 dysfunctional myeloid tumour cells in acute myeloid leukemia (AML). However, H22(scFv)-ETA' has only been produced in shake flasks, a process that cannot feasibly provide sufficient amount for clinical trials and ultimately commercial scale. This study aims to optimise productivity of the H22(scFv)-ETA' fusion protein. Thereafter, the scale up of the reactor system used from a 300 mL stirred-tank reactor (STR through the 5 L scale) to a 30 L STR will be undertaken. H22(scFv)-ETA' will be recombinantly expressed in the periplasmic space of E. coli Bl21 (DE3) under osmotic stress, purified by ion-affinity chromatography and size exclusion chromatography. To achieve scale-up of batch and fed-batch production processes the mass transfer coefficient (kLa), aeration rate (Q) and the impeller agitation speed (N) will be adjusted to ensure a constant kLa at the 300 mL, 5 L and 30 L scales. BL21 (DE3) culture kinetics at the three scales will be compared in terms of volumetric productivity (QP), specific (qp) H22(scFv)-ETA' production rates, production yield (Yp/s), specific growth rate (μ) and substrate utilization rate for kLa values that provide effective mixing and aeration at each scale. Therapeutic efficacy will be evaluated by several biological assays including binding assays, flow cytometry assays and the cytotoxicity assessed by annexin V bioassay. It is envisaged that, by characterising the key engineering parameters affecting performance, we will achieve an increased production of functional H22(scFv)-ETA' and maintain this at 30 L. Development of a successful scale-up production of H22(scFv)-ETA' will thus be a major achievement, enabling insights of a process to be established at commercial scale.

WATER & THE ENVIRONMENT

Mixed Microbial Bioprocess Systems for Bioremediation of Mine-Impacted Effluent Waters

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Keywords: Thiocyanate, cyanide, bioprocess, bioremediation, biofilm

Many industrially-relevant bioprocesses, including mine-impacted waste water bioremediation, are comprised of complex mixed microbial communities operating synergistically to catalyse a particular process. In most cases the overall process itself is well characterized; while, the microbial consortia implicated in these biological processes are not as well understood. Traditionally, culture-based methods have been used to characterize these complex microbial communities, however, molecular analyses have shown that 0.001-1% of the microorganisms within a given sample can be isolated using conventional approaches. Increasingly, genomic methods are being used to investigate processes in wastewater treatment to identify key microbial species and, thereby, inform the rationale design and operation of these bioremediation systems. Analysis of the total genomic DNA, or metagenome, of a particular bioprocess system can provide insight into the community composition and relative abundance of individual species, as well as the metabolic potential of the microorganisms contained within these intricate microbial consortia. In the context of bioprocess engineering, this information can be used to better our understanding of the microbial communities associated with these processes. Applying this knowledge will inform our ongoing efforts to enhance the processes being investigated, through targeted reactor design and/or selection to favour the proliferation and activity of specific microorganisms catalysing the reactions of interest. The initial phase of these studies involves defining the key microbial species, within these reactor systems, and how these target microorganisms respond, in terms of abundance profiles and metabolic activity, to process-relevant perturbations. This information is applied to assess the effect of process operating conditions on system performance and may be followed by the selection of an alternative reactor configuration(s) and/or rationale changes to the operation.

Reactor Selection for Biodegradation of Thiocyanate and Cyanide Based on Microbial Community Characteristics

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Keywords: Thiocyanate, cyanide, bioprocess, bioremediation, biofilm, bioreactor design

Thiocyanate and cyanide are found in a range of industrial wastewater effluent streams, including those from the leaching of gold from sulphidic ores. The microbial degradation of cyanide and thiocyanate in these streams has been demonstrated at various scales and has been shown to produce ammonia and sulphate, which are less toxic. A collaboration between UC Berkley and CeBER has conducted extensive metagenomic analysis on a complex mixed culture in bioreactors degrading thiocyanate and cyanide, resulting in an enhanced understanding of the community and its metabolic potential. The microbial communities were found to be more diverse than previously reported and possessing a wide range of metabolic capacities.

This project further investigates the characteristics and metabolism of the assortment of microbial communities present in a thiocyanate- and cyanide- degrading bioreactor, and uses the findings to propose improvements to current reactor operation. For instance, the study explores biomass retention in the bioreactors to increase microbial concentration in a single reactor, thus improve degradation rates and reduce required reactor volumes. The retained microorganisms also form biofilms which have been reported to improve system resilience by withstanding unexpected operation changes better than unattached biomass.

In addition to thiocyanate- and cyanide- degrading microorganisms, the microbial community was also found to contain microorganisms with the potential for nitrification and denitrification. This opens up the possibility of further treatment of the effluent stream to remove nitrogen which may cause eutrophication if discharged into environmental water sources. Ongoing work in this study considers reactor operation that can maximise microbial capacity to result in less polluted water.

Knowledge of microorganisms present in bioreactors not only uncovers limits of reactor performance, but also opens up the possibility of discovering ways to boost reactor performance in different operating contexts. This project intends to show how bioprocess design that deliberately examines and takes advantage of the functions of present microbial communities results in processes that are economically, environmentally and operationally sustainable.

An integrated semi-passive bioprocess capable of simultaneous sulphate reduction and sulphide oxidation for the treatment of low volume acid rock drainage

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Keywords: sulphate reduction, partial sulphide oxidation, semi-passive bioprocess

Long-term formation of ARD and contamination of freshwater represents the biggest environmental challenge facing the world after climate change. In the context of South Africa (SA), the impact of ARD has been exacerbated by an extended country-wide drought. As freshwater becomes increasingly scarce, the preservation and management of existing water resources becomes strategically important to ensure water security. In SA, the primary focus on remediation of ARD-contaminated water has been based on established active technologies. These approaches have proven to be costly, leads to secondary challenges and are not applicable for the remediation of lower volume discharges. Mostly overlooked, ARD discharge from diffuse sources, associated with coal mining in SA, will have a far greater impact on the environment, due to the sheer number of deposits and geographic distribution over rural areas, than those originating from point sources. Semi-passive ARD treatment systems present an attractive alternative for addressing these low-flow sources, with lower capital and operational costs than active systems and better control and greater predictability than conventional passive systems. Research conducted at the Centre for Bioprocess Engineering has resulted in the successful development of a hybrid semi-passive bioprocess capable of simultaneous biological sulphate reduction and partial sulphide oxidation with the added value recovery of elemental sulphur that can be used in agriculture for soil amelioration. The hybrid process relies on the activity of a diverse compliment of sulphate reducing and sulphide oxidising microbial communities within a single unit of operation that enable effective neutralisation, sulphate removal and sulphide management through the recovery of elemental sulphur from sulphate-rich ARD waste streams. Therefore addressing the major toxicological concerns associated with ARD. The final treated effluent is regarded safe and can be discharged into the environment. The process offers a sustainable cost effective solution for the treatment of low volume ARD discharges and can be applied in remote areas devoid of infrastructure and electricity. Ultimately future implementation of this process will contribute to addressing the long term nature of ARD contaminated water and its impact on the environment as well as provide a sulphur product that can be used for agriculture.

Biological Sulphate Reduction: Linking the Performance and Microbial Ecology of a Number Reactor Systems Using Applied Metagenomics

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Keywords: Biological sulphate reduction, sulphate reducing bacteria, metagenomics, 16S rRNA sequencing, whole-genome metagenomic sequencing

Biological sulphate reduction (BSR), has shown great promise as a sustainable low-cost remediation strategy for low-flow ARD effluents. This process is catalyzed by sulphate reducing bacteria (SRB) which use sulphate as the terminal electron acceptor in the oxidation of organic compounds, resulting in the formation of sulphide and carbonate. The produced sulphide forms stable precipitates with heavy metals or can be converted to elemental sulphur and the carbonate aids in neutralization of the solution, making these microorganisms an ideal biocatalyst for the treatment of low-flow ARD effluents. However, a thorough understanding of the microbial community structure of SRB in BSR reactor systems and the identification of SRB integral to robust reactor performance have received little attention in existing literature. In this study, three continuously operated reactor configurations, namely a continuous stirred tank reactor (CSTR), an up-flow packed bed reactor (UAPBR) and a linear flow channel reactor (LFCR), are operated in duplicate and supplemented with either acetate or lactate as an electron donor, respectively. The CSTRs support a planktonic microbial community whereas solid support matrices are incorporated into the UAPBR and LFCR to enhance biomass retention through the generation of attached microbial communities. The variation in flow patterns between the three reactor configurations, the supplementation of different electron donors and the inclusion of support matrices generates a range of distinct environments within these systems. These environments are anticipated to act as selective pressures on microbial growth and persistence, allowing for the development of dissimilar microbial communities. The performance and microbial ecology of these reactor systems are assessed as a function of hydraulic residence time. The reduction in hydraulic residence time acts as a further selective pressure, intended to reduce the microbial diversity of these systems and enabling the identification of key SRB. Metagenomic DNA sequencing including 16S rRNA gene and whole-genome sequencing are employed to resolve the complex microbial communities associated with these reactor systems. The structure of the respective microbial communities are overlaid upon collected system performance data, from the continuous operation of these reactor systems, allowing for the link between microbial community structure and system performance to be identified.

Characterisation and Development of a Coupled Linear Flow Channel Reactor for Simultaneous Sulphate Reduction and Sulphide Oxidation

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Keywords: Biological sulphate reduction, acid mine drainage, sulphide oxidation, bioremediation, semi-passive

Acid mine drainage (AMD) is an increasingly major threat to global freshwater supplies. Biological sulphate reduction (BSR) which occurs via the action of sulphate reducing bacteria (SRB) has shown great remediation potential of waters high in sulphate ions and is amenable to slow-flow sources of AMD. This study involves optimization of a semi-passive linear flow channel reactor which integrates BSR, with partial oxidation of the sulphide produced to elemental sulphur, a value-added product. The system being used has previously shown decreases in sulphate concentrations of up to 80%. However, one of the key challenges associated with sustainable BSR, particularly for remote mining locations, is the cost of the electron donor. Various electron donors in the form of volatile fatty acids (VFAs) are being used in this study, namely lactate, propionate and acetate. Acetate and propionate have both been shown to be present in anaerobic digestate and therefore are relevant as potential low-cost substrates. The project revolves around the fact that the higher chain VFAs, lactate and propionate are generally only partially oxidised to acetate under biosulphidogenesis. This results in inefficient use of the electron donor as well as in effluent with a high organic content. Studies however indicate that different microbial populations develop under different electron donors. Therefore, a coupled reactor system will be trialed, initially utilizing lactate since it has been well studied in this system. A first reactor unit which has selected for lactate-utilizing SRB will be fed with lactate and coupled in series to a second reactor unit specifically colonized with acetate-utilizing SRB. This second reactor is therefore expected to polish off the residual sulphate via acetate oxidation resulting in a fit-for-purpose effluent. Propionate has been little-studied in this space and will be first studied in a single reactor system. The overall objective is to investigate the balance between good sulphate reduction rates and the most efficient utilization of the relevant electron donors.

ALGAE

Algal Biotechnology as a Platform to Valorise Various Industrial Waste Streams in South Africa

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Keywords: Microalgae, bioremediation, industrial effluent, carbon capture

Currently there is an increasing demand in terms of energy requirements, as most industries are heavily reliant on the utilization of diminishing fossil fuels and water. Therefore, there is an urgent need to explore sustainable options of generating energy. Microalgae have emerged as a promising platform to address some of the environmental issues in South Africa. Although most algal studies to date have focused on the growing field of biofuel production, microalgae have concomitant potential to bioremediate aqueous effluent streams, particularly those rich in nitrogen and phosphorus. Their ability to proliferate effectively in nutrient rich streams as well as sequester carbon dioxide (CO_2), provides a platform for producing high value products or for utilization as renewable energy resources.
The algal biotechnology platform at CeBER conducts research across these focal areas. Aspects of our research is focussed on the production of high value compounds such as pigments while we also hold a strong focus on rapid CO_2 assimilation and the potential of microalgae in bioremediating various municipal and industrial effluents in South Africa, with an objective of producing fit-for-purpose water and other valuable products. Our approach centres on screening and characterization of the inhouse, and continually extending, microalgal library for indigenous strains that can assimilate carbon rapidly, utilize various nitrogen sources, produce algal biomass for commodity product use and maximise productivity of key high value algal products. Further we have developed potential for targeted algal isolation and selection and for genetic manipulation. This is balanced by engineering developments in reactor design and microalgal separations, as well as scale up considerations.
Applications include development of the algal biorefinery producing a balance of high value products such as pigments and nutraceuticals, commodity, feed and bioenergy products, treating nitrogen rich waste water streams, using metal assimilating microalgae in the treatment of effluents with high concentrations of heavy metals. Furthermore, the CeBER algal platform is also conducting research to better understand the mechanisms of CO_2 capture by various indigenous microalgal strains. This work is aimed at identifying strains with high CO_2 uptake rates. The technologies coming out of this platform will make a great contribution towards maximizing the performances of the current waste water treatment systems in South Africa while simultaneously opening a window for various other 'green' products.

Design of a Novel, Scalable, Surface aerated, Photobioreactor

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Keywords: Photobioreactor, surface aerated, algae.

This project focuses on the design of a new surface aerated, closed photobioreactor to be used for, but not limited to, algal cultivation. The wave bioreactor has been shown to achieve comparable and sometimes even better mass transfer than several other reactors that are commonly used for algal production. However, this reactor suffers from scalability issues with a maximum reported working volume of 1000 to 2000 L. Therefore, a new reactor without the same scalability issues but which achieves the same performance needs to be designed. The new reactor seeks to mimic the liquid motion in the wave reactor using a new mechanism. The new reactor has been designed based on the best mass transfer conditions shown in previous experiments, under low nitrogen conditions. Tests are currently carried out in the wave reactor to validate that the best mass transfer conditions previously shown hold under high nitrogen conditions. Following the construction of a new reactor, its performance in terms of supporting microalgal growth, will be assessed. Thereafter, its scale up will be addressed.

Carbon Dioxide Capture Using Microalgae in Different CO_2/HCO_3- Ratios

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Keywords: Microalgae, carbon capture, carbonic anhydrase, dissolved inorganic carbon.

There is worldwide concern about global warming caused by emission of greenhouse gases, mainly CO_2. There is an urgency to mitigate CO_2 emission. This is being addressed using CO_2 capture, storage and cycling methods. Microalgae have been identified for potential use in CO_2 capture. Productivity of microalgae in open pond systems prevalent in large-scale operations is typically limited by mass transfer of CO_2. Mass transfer of CO_2 to support algal growth can be divided into gas-liquid transfer and the subsequent CO_2 uptake by algae cells. Carbon dioxide is known to speciate into three inorganic carbon species, namely CO_2, bicarbonate (HCO_3-) and carbonate (CO_3^{2-}), based on the pH of the media. The mechanism of CO_2 uptake by algae cells depends on the carbon species present in the media. Algae can take up CO_2 and HCO_3- whilst CO_3^{2-} is not utilized. While CO_2 uptake rates are reported for some algal species, inconsistent reactor types and operating conditions make comparison difficult, to select the species that efficiently take up CO_2. This research involves development of a novel methodology to measure CO_2 uptake by microalgae in both CO_2-rich and HCO_3-rich solutions and to identify algae species that take up the most CO_2. It was hypothesized that screening for microalgae species to use in CO_2 capture can be implemented based on the activity of periplasmic carbonic anhydrase (CA), a key enzyme in CO_2 uptake. The outcome of this research is the fundamental understanding to form the basis for the optimization of CO_2 uptake by microalgae. Furthermore, the methodology developed for measuring CO_2 uptake will be useful in the screening and identification of key algae species for sustainable application in large scale CO_2 capture.

Re-Purposing of Nitrogen in South African Wastewaters Using Algal Biotechnology for Value Addition

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Keywords: Eutrophication, wastewater, microalgae, remediation

The eutrophication of water sources, especially by high nitrogen and phosphate containing compounds, causes pollution that has become a worldwide problem, threatening the environment and human health. Wastewaters containing high quantities of these nutrients may support the growth of aquatic organisms such as algae, leading to uncontrolled rapid growth which results in eutrophication. Currently in South Africa, where infrastructure exists, wastewaters are treated by the nitrification/denitrification processes for nutrient removal; however, where the nutrients in processed wastewater are not eliminated in totality due to sub-optimal operation or overloading of the system, eutrophication may still occur. Amending the already existing infrastructure to improve the remediation of wastewater is costly and the existing infrastructure is already an economical burden to many industries and communities. Therefore, there is a need to look for efficient alternatives. One of these is the use of microalgae as a water remediation tool. The cultivation of these aquatic species has the added benefits of requiring only low-cost infrastructure, producing algal biomass that may be utilized for bioproduct recovery or energy production or both, and contributing to carbon mitigation through CO_2 assimilation. Where nutrients are only present in trace amounts in wastewaters, they may still have the capacity to support the growth of microalgae. Furthermore, these may be combined with more concentrated nutrient waste streams to create a balanced nutrient medium to support algal growth with associated nutrient removal from wastewaters. This opens an opportunity to explore the possibility of resource efficiency with the re-purposing of contaminants in the water through production of algal biomass and associated value-added products while remediating the water for reuse. Investigations are focused on remediating nitrogen rich wastewater streams and thus nitrogen polluted wastewaters will be prioritized. Selection of algal species will be based on their suitability to remediate specific wastewater streams based on their ability for rapid nitrogen assimilation and to scavenge the different nitrogen forms predominating different wastewaters to very low levels.

Phycoremediation of Heavy Metal Contaminated Water Streams for the Production of Fit-For-Purpose Water and Value-Added Bioenergy

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Keywords: Microalgae, heavy metal, bioremediation, bioenergy

The discharge of wastewater streams containing heavy metals has increased sharply since the beginning of the Industrial Revolution. Anthropogenic activities such as plastic, textile, mining and metal smelting generate large volumes of this waste, some of which is discharged into the water sources, particularly in developing countries. The toxicity of heavy metals to plant and animal life has provided impetus for research focused on the remediation of these waste water streams prior to their contaminating environmental water sources. Biological remediation by the use of microalgae has been identified as an effective means of removing contaminants from water streams and is favoured over its chemical and physical counterparts due to its process simplicity, reduction in secondary pollutants and potential for economic viability. One of the key factors in determining the feasibility of using microalgae for heavy metal water remediation is the utilisation of the resultant heavy metal laden biomass and its associated economic benefit. This biomass cannot be used in the agricultural sector or for animal consumption due to the presence of toxic heavy metals. However, microalgae have been shown to have a variety of industrial applications for the production or co-production of biofuels, including biogas (anaerobic digestion and catalytic hydrothermal gasification), biodiesel (transesterification) and biocrude oil (hydrothermal liquefaction). This study aims to identify microalgal species with potential for both heavy metal remediation by uptake and biomass production using a high-throughput screening method. Microalgal species which present these traits are characterised in terms of their growth potential and biomass generation in the presence of heavy metal contaminated water, allowing selection on the basis of volumetric heavy metal uptake rate. In addition to this characterisation, the determination of the cellular location of the heavy metals removed from the water will inform the selection of the most applicable biofuel production process which allows concomitant concentration or recovery, or both, of the heavy metals.

Extracting Phycocyanin from *Spirulina*

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Keywords: *Spirulina*, Phycocyanin, ATPS, Preciptation

Phycocyanin is a blue pigment that is highly desirable as one of very few natural blue pigments for use in the food and cosmetic industries. In addition to its striking colour, phycocyanin also has beneficial nutritional qualities including immune enhancement, anti-inflammatory and anti-oxidant properties and is thus valuable as a food additive or health supplement. It also has interesting fluorescent properties and is used in immunodiagnostic and analytical applications.
The project aimed to develop an innovative process to produce high quality phycocyanin from *Spirulina* in a globally competitive manner within South Africa. An optimised downstream processing train was demonstrated which produces a food or cosmetic grade phycocyanin product from a feed of either fresh wet or previously dried *Spirulina*. After cellular disruption to release the phycocyanin pigment-protein complex into solution, aqueous two phase extraction using PEG and maltodextrin was used as a first purification step to separate the phycocyanin from other soluble contaminants. This is followed by a train of three ammonium sulphate precipitation stages to improve the purity of the phycocyanin product and further reduce the microbial content to the required levels.
The purification route is currently in the process being patented (United Kingdom Patent Application No. 1713293.7).