Novel biochemical and catalytic microwave-assisted methods for the synthesis of bio-butanol

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DECLARATION

I, **Busiswa Ndaba,** hereby declare the thesis entitled **“Novel biochemical and catalytic microwave-assisted methods for the synthesis of bio-butanol”** is my work. It has not been submitted before for any degree or examination in any other University. All sources of information have been specifically acknowledged by means of references in each chapter.

Signed.................................at..on................................this day...........................of......

PREFACE

Thesis format

The format of the thesis is written according to the general academic rules stipulated by the **North-West University:**

Rule A.7.5.7.4 states “If a student is allowed to present a dissertation or mini-dissertation in the form of (a) published research article(s) or (an) unpublished manuscript(s) in article format, and if more than one such manuscript or article is used, the dissertation or mini-dissertation must still be presented as a unit, supplemented by an overarching problem statement, a focused literature analysis and integration, together with a summarised concluding discussion”.

Rule A.7.5.7.5 states “If any research article or manuscript, and/or an internationally examined patent, involving more than one author and/or inventor is used the student must obtain a written statement from each co-author and/or co-inventor stating that such co-author and/or co-inventor consents to the use of the research article or manuscript and/or patent for the intended purpose and indicating the extent of each co-author’s and/or co-inventor’s share in the research article or manuscript and/or patent concerned”.

Rule A.7.5.7.6 states “If any co-authors have been involved the student must mention that fact in the preface and must have the statement of each co-author inserted after the preface in the mini-dissertation or dissertation”.

Numbering and referencing style

It should be noted that the style of referencing, figure and table format of the three articles were altered from their original published format in order to adopt consistency for full thesis submission. The content of the manuscripts were modified from the submitted and/or published versions to accommodate examiners comments.

STATEMENT FROM CO-AUTHORS

To whom it may concern

This section complies with rules *A5.4.2.8 and A.5.4.2.9* of the academic rules, as stipulated by the North-West University.

Publications involving co-authors were used, and permission from these authors has been obtained. The listed co-authors hereby give consent that **Busiswa Ndaba** may submit the listed manuscripts under the “list of publications” section as part of her thesis entitled: **Novel biochemical and catalytic microwave-assisted methods for the synthesis of bio-butanol** for the degree *Doctor of Philosophy in Chemical Engineering* at the North-West University.

Co-author

Signed.................................at..on...................................this day...........................of......

Co-author

Signed.................................at..on...................................this day...........................of......

LIST OF PUBLICATIONS

Paper I (published)

Ndaba, B., Chiyanzu, I., Marx, S. 2015. *n*-Butanol derived from biochemical and chemical routes: A review. *Biotechnology Reports*. 8:1-9. ***Cited by 81***

Paper II (published)

Ndaba, B., Chiyanzu, I., Marx, S. 2015. Direct fermentation of sweet sorghum juice by *Clostridium acetobutylicum* and *Clostridium tetanomorphum* to produce bio-butanol and organic acids. *Biofuel Research Journal*. 6:248-252. ***Cited by 5***

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Marx, S. and Ndaba B**.** 2019. Rapid microwave-assisted liquid phase conversion of bio-ethanol to n-butanol over a heterogeneous catalyst. *Biofuels*. 19:1-8

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Ndaba, B., Chiyanzu, I., Marx, S. Bio-butanol fermentation using *Clostridium acetobutylicum* and *Clostridium tetanomorphum* in modified bioreactor flasks. European Biomass Conference and Exhibition, Vienna, Austria, 1-4 June 2015 (Poster presentation).

Ndaba, B., Marx, S. Rapid microwave-assisted bio-ethanol conversion to *n*-butanol using Nickel on alumina as a catalyst. Renewable and Sustainable Energy Postgraduate Symposium, University Of Fort Hare, Alice Campus, South Africa, 5-6 September 2016 (Poster presentation).

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"We rejoice in our sufferings, because we know that suffering produces perseverance; <u>perseverance,</u> <u>character;</u> <u>and</u> <u>character,</u> <u>hope.</u> And hope does not disappoint us, because God has poured out His love into our hearts through the Holy Spirit, whom He has given us"

ROMANS 5:3

EXECUTIVE SUMMARY

Biofuels such as bio-ethanol, bio-butanol and biodiesel are attractive fuel substitutes to negate the negative effects of fossil fuel use. Bio-butanol, in particular, is of interest because of its properties, such as; low volatility, less ignition problems and high intersolubility. These properties resemble that of petroleum fuel, making it a drop-in fuel that is more acceptable to internal combustion engines than bio-ethanol. Bio-based butanol is mostly produced through fermentation processes with relatively low yields and high cost. If bio-based butanol is to be used as a bulk fuel to replace petroleum, methods need to be developed to lower production and fuel costs. Therefore, in this study, alternative synthesis methods for lowering the cost of bio-based butanol through biological and chemical pathways were investigated and compared.

An extensive literature review reported on the butanol production protocols covering both traditional Acetone-Butanol-Ethanol (ABE) fermentation and catalytic upgrading of bio-ethanol to *n*-butanol. The literature review was published as a review article (B. Ndaba, I. Chiyanzu, S. Marx. 2015. *n*-Butanol derived from biochemical and chemical routes: A review, *Biotechnology Reports*, 8: 1–9, https://doi.org/10.1016/j.btre.2015.08.001). Given the shortcomings of ABE fermentation, such as long fermentation times and difficulty in obtaining high butanol yield due to co-production of acetone and ethanol, the review highlighted the chemical process route as the most prudent method for producing bulk bio-based butanol. An ABE fermentation process of sweet sorghum juice was investigated as a comparative baseline study using different inoculum concentrations (3, 5, 10 %v/v). A single culture of *C. acetobutylicum* at culture loading of 10 %v/v resulted in a high bio-based butanol concentration of 6.49 g/L (0.06 g/g of sweet sorghum juice) after 96 hrs of fermentation, while fermentation with *C. tetanomorphum* fermentation produced a bio-based butanol concentration of 2.66 g/L (0.02 g/g of sweet sorghum juice) at a 5 %v/v inoculation. The baseline study showed that *C. acetobutylicum* could enhance bio-based yield compared to *C. tetanomorphum*, but, the butanol yield was however still low. The findings from the baseline study were published as a research article (B. Ndaba, I. Chiyanzu, S. Marx. 2015. Direct fermentation of sweet sorghum juice by *Clostridium* *acetobutylicum* and *Clostridium tetanomorphum* to produce bio-butanol and organic acids, *Biofuel Research Journal*, 6: 248-252, https://www.biofueljournal.com/article_9690.html, DOI: 10.18331/BRJ2015.2.2.7). It was concluded from these results that high enough butanol yields cannot be achieved with current biological pathways to meet fuel demands at an affordable price. Therefore, chemicals pathways for the production of bio-based butanol from bioethanol was investigated.

Bio-based butanol production through microwave-assisted catalytic conversion of bio-based ethanol was investigated using three catalysts, i.e. magnesium oxide (MgO), platinum on alumina (Pt/Al_2O_3), and nickel on alumina (Ni/Al_2O_3). All catalysts were characterised in terms of physical properties using transmission electron microscopy (TEM), Brunauer-Emmett-Teller (BET), Powder X-ray diffraction (P-XRD) and thermogravimetric analysis (TGA). The reaction was conducted in an industrial microwave reactor according to a 2-level factorial design with reaction temperatures (200°C and 250°C), catalyst loading (1 and 5 wt.%) and reaction times (30 and 60 min) as manipulated variables. Catalyst performance was compared by measuring and comparing the bio-based butanol selectivity and bio-based ethanol conversion. The Ni/Al_2O_3 catalyst was shown to have the best performance in terms of bio-based butanol selectivity (58%) and bio-based ethanol conversion (20.8wt.%) compared to the Pt/Al_2O_3 catalyst which achieved only a 9.2wt.% ethanol conversion in 30 min with an 11.5% selectivity towards *n*-butanol produced. The MgO catalyst produced mostly acetaldehyde at a concentration of 140 g/L (0.13 g/g of bio-ethanol) as a main product at an ethanol conversion of 10.7 wt.%. Therefore, the Ni/Al_2O_3 catalyst was selected for further investigations. The effect of residence time on bio-based butanol yield using Ni/Al_2O_3 catalyst was investigated at residence times between 5 and 60 min. Product yield and distribution was determined using high performance liquid chromatography (HPLC). The highest *n*-butanol concentration of 161 g/L (0.22 g/g of bio-ethanol) was achieved at an *n*-butanol selectivity of 60% and ethanol conversion of up to 76.6 wt.% at a residence time of 60 min. Conversion was shown to be higher in a shorter time (60 min) compared to the ABE fermentation process (96 hrs).

This study introduced a new microwave-assisted method for the rapid conversion of bio-based ethanol to bio-based butanol. Overall, a microwave-assisted system for *n*-butanol production offers advantages of shorter reaction time and lower reaction temperatures compared to conventional reaction methods and ABE fermentation. Furthermore, bio-based ethanol can be produced from a wide range of feedstock, including biomass which does not compete with food or feed. In addition, the use of agricultural residues to produce ethanol for bio-based butanol production by the method presented in this study, ensures that no land use change is involved, resulting in a much more environmentally friendly fuel production chain. This study presents a new and innovative method for bridging the gap towards environmentally responsible and economically sustainable biofuels production.

Keywords: Sweet sorghum juice, *Clostridium* species, bio-ethanol, catalyst, microwave, *n*-butanol

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NOMENCLATURE

Symbol/abbreviation	Description
g/L	gram per litre
kg	kilogram(s)
w%	weight percentage
μL	micro litre
μm	micro mitre
Å	Angstrom
λ	Lambda
β	Beta
ϴ	Theta
v/v	volume per volume
^oC	degrees celsius
m^2/g	metre squared per gram
MJ/kg	megajoules per kilogram
g/g	gram/gram
kV	kilovolt
$^oC/min$	degrees celsius per minute
min	minute(s)
hr	hour(s)

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction and motivation

The environmental and financial burden of mitigating the negative impact of climate change has become an important global issue. The burning of petroleum and coal creates large amounts of greenhouse gas emissions that trap heat in the earth's atmosphere contributing to significant heating of the surface (global warming). Climate change is estimated to negatively affect and compromise the supply of adequate fresh water for domestic purposes and agricultural production worldwide. It is estimated that agricultural yields in South Africa could drop by approximately 50% with arid land increases of 5 to 8% (COP 17, 2011).

South Africa depends on crude oil imports and local coal reserves to meet the country's fuels and chemicals demand. Oil is a major component of South Africa's energy mix, accounting for approximately 17% of South Africa's primary energy needs (SANEA, 2016). Approximately 86% of crude oil is imported from Saudi Arabia, Iran and Nigeria to meet nearly 64% of the local demand for liquid fuels. In addition, South Africa is highly dependent on coal as an energy source, with about 77% of the country's primary energy needs being provided by coal; 62% for electricity generation and 23% for petrochemical industries (DoE, 2018). However, coal is not a renewable energy resource and coal use is responsible for 55.1% of South Africa's GHG emissions (DEA, 2016). These are convincing reasons to be concerned for future GHG emissions reduction targets and energy security.

It is crucial for the country to find a sustainable path to ensure adequate energy production to reduce oil and coal dependency and negative climatic conditions. Therefore, alternative biofuels such as biodiesel, bio-ethanol and bio-butanol, have been identified and investigated to reduce fossil fuel dependency (Pereira et al., 2015; Rom and Friedl, 2016). The first biofuel to be extensively explored was bio-ethanol, with Brazil having the leading pilot scale plants and blending ethanol in low percentages (E10 and E2) with petroleum

(Jordison, 2016). To be able to achieve higher blending ratios, some new compounds should be investigated. One possible method is to convert ethanol to *n*-butanol; unlike ethanol, butanol can be used as a drop-in fuel in internal combustion engines without blending (Zhu et al., 2016; Xie et al., 2016). Butanol has several other advantages over ethanol, such as higher energy content due to higher number of carbon atoms, a higher octane number (96 RON) and lower volatility (Dziugan et al., 2015).

To date, there are three major consumers of bio-based butanol; i.e. the Asia-Pacific region (47%), North America (24%) and the European region (25%). Bio-based butanol production has consistently increased annually since 2010 (Galadima & Muraza, 2015; Bharathiraja et al., 2017). Global production of butanol exceeds 4.5 billion litres annually and the valued market is targeted for lower cost butanol production. According to Bharathiraja et al. (2017), the estimation of butanol market expansion is at 3% per year.

Butanol (butyl alcohol) is a four-carbon alcohol largely used as a solvent, chemical intermediate and extractant in cosmetics and pharmaceutical industries. This alcohol has four distinct isomeric structures, i.e. *n*-butanol ($n\text{-}C_4H_9OH$), *sec*-butanol ($sec\text{-}C_4H_9OH$), *iso*-butanol ($iso\text{-}C_4H_9OH$) and *tert*-butanol ($tert\text{-}C_4H_9OH$) (Yang et al., 2007; Gu et al., 2010; Weber & Sung, 2013) and can be derived by means of either biological or chemical processes. The former is referred to as bio-based butanol, because it is produced from biomass. The biological method commonly known as the ABE process makes use of natural and renewable biomass such as sweet sorghum, sugarcane, and maize to produce acetone, butanol and ethanol in the presence of microorganisms from the Clostridia class (Xiong et al., 2016).

These ABE products can be produced from different parts of biomass, these include; starchy (grains), lignocellulosic (hemicellulose and cellulose), or through liquid extraction (juice/syrup). The production process from starch normally involves saccharification to form simple sugars which can be fermented to acetone, butanol and ethanol. A lignocellulosic material is initially pre-treated to delignify the plant materials and make the cellulose and hemicellulose accessible for enzymatic saccharification to fermentable sugars. The juice/syrup can be directly fermented to form ABE products.

During ABE fermentation, *Clostridium* bacteria goes through an initial growth phase, where acids (e.g., acetic and butyric) are produced from sugar molasses or starch followed by a solventogenic phase where the acids are converted into solvents and alcohols (e.g., acetone, butanol, and ethanol) (Jones and Woods, 1986). However, the use of bacterial strains during fermentation is very difficult to control, because of environmental changes and inhibitors present during fermentation. In addition, butanol concentration higher than 10 g/L inhibits cell growth and limits further butanol production. As a result, butanol concentrations in ABE fermentative broths are usually below 13 g/L (Ramey & Yang, 2004). Therefore, more efforts are required in improving butanol concentrations by investigating different feedstock and bacteria (Zhu et al., 2016; Klasson et al., 2018).

Considering the challenges associated with the ABE process, there is growing interest in producing bio-butanol through condensation of bio-ethanol over a suitable catalyst. Bio-ethanol conversion to bio-butanol has been achieved using different catalysts to improve selectivity (Tsuchida et al., 2008). The chemical conversion of ethanol to butanol results in high butanol yields (62%) in a shorter time than fermentation (Ghaziaskar & Xu, 2013). Metal oxide nanoparticles are of industrial interest as catalysts for butanol production. Their basicity-acidity and compositional properties allow for catalysis of a wide range of chemicals, including light alcohol condensation into higher alcohols (Oswald et al., 2011).

Metal oxide catalysts such as Ni, Pt, Mg on alumina have been reported (Ndou et al., 2003, Riittonen et al., 2012) to be some of the most promising catalysts for conversion of ethanol to butanol. The reaction is usually carried out in the gas phase in either a batch (Riittonen et al., 2012; Sun et al., 2016) or continous stainless steel reactor with temperatures ranging from 300 to 450 ^{o}C for 6-12 hrs (Dziugan et al., 2015). In view of the energy consumption associated with high temperatures and long reaction times, ways of conducting the reaction at lower temperature and in the liquid phase at both high ethanol conversions and butanol selectivity are needed.

Several researchers have investigated the use of microwave heating to promote chemical reactions by reducing reaction time and improving product yield (Mirzaei and Neri, 2016;

Eslami et al., 2017). In order to counter the low butanol yields associated with ABE fermentation and the long reaction times as well as high temperatures associated with traditional ethanol condenstation to butanol, a rapid microwave-assisted method to produce butanol from ethanol was developed in this study.

1.2 Aim and objectives of the study

The aim of the study was to investigate a new approach for production of bio-based butanol that supersedes the limitations of the current ABE process and improves the conventional catalytic process for production of *n*-butanol from ethanol.

The objectives were:

- To investigate microorganisms (*Clostridium acetobutylicum* and *Clostridium tetanomorphum*) that can improve bio-based butanol yield from the ABE process. This objective was achieved by:
    - identifying and quantifying sugars found in sweet sorghum juice,
    - evaluating pretreatment conditions for long term storage of the juice,
    - optimizing the concentrations of *C. acetobutylicum* and *C. tetanomorphum* for improved butanol production.
- To investigate an alternative microwave heating method for bio-based butanol production using MgO, Pt/Al_2O_3, and Ni/Al_2O_3 as catalysts. This objective was achieved by:
    - investigating different catalyst loading for improved butanol selectivity,
    - investigating the optimal reaction temperature for improved butanol selectivity,
    - investigating the effect of reaction time on ethanol conversion and butanol selectivity using a selected catalyst under optimal microwave reaction conditions.

1.3 Scope of the study

This thesis is compiled from three peer-reviewed publications (Chapters 2-4) generated during the course of the PhD study. A detailed scope concerning the experimental chapters is summarized in the block diagram (see Figure 1).

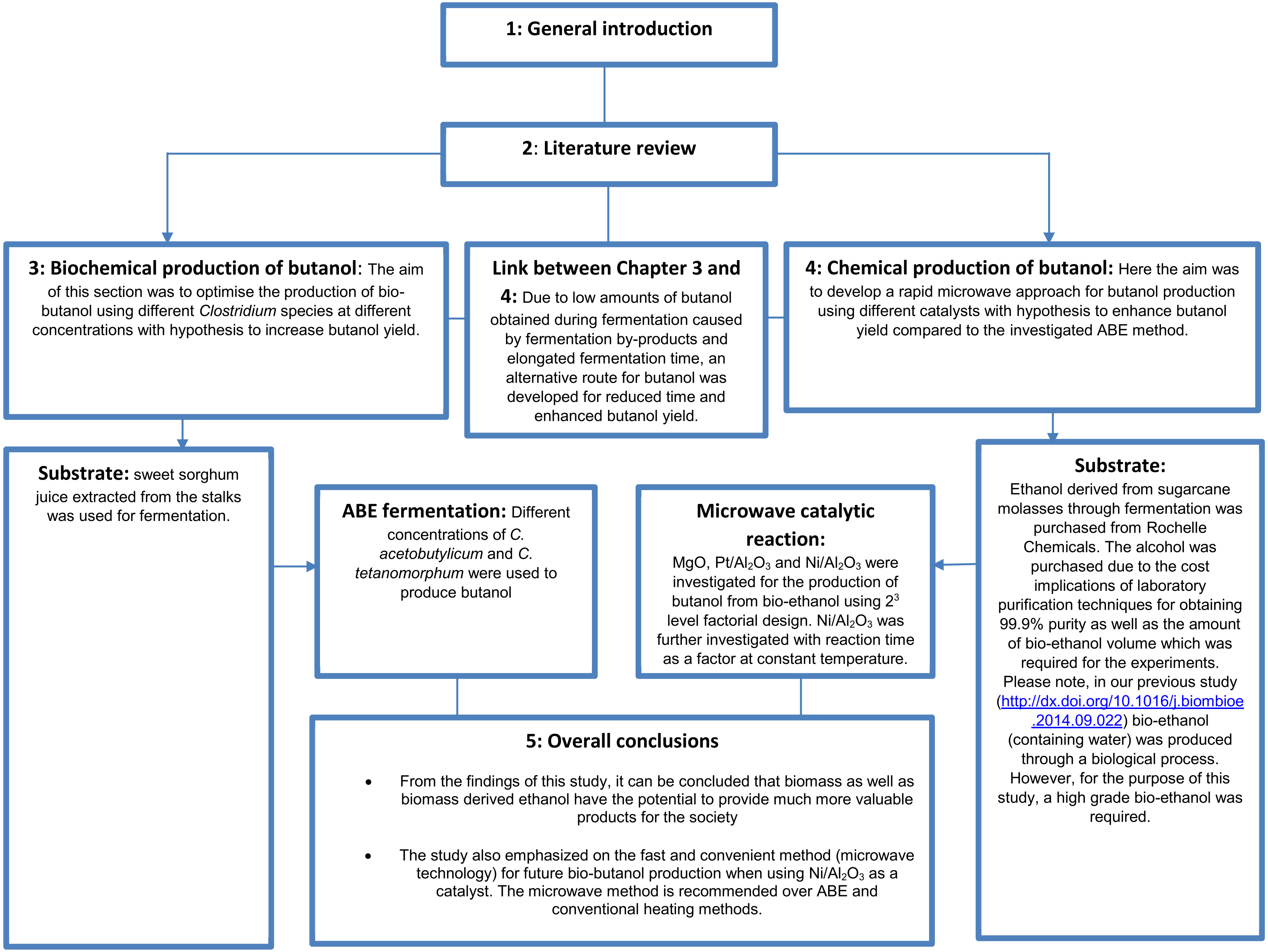
Chapter 1- provides the introduction of bio-butanol as a drop-in fuel. This includes primary fundamentals, application and technologies involved during the production.

Chapter 2 - (peer-reviewed published paper) this review dealt with relevant butanol production methods, emphasising the merit of the current preparation processes. An extensive survey was focused on the reported production protocols based on two processes, namely, ABE fermentation and catalytic conversion of ethanol to *n*-butanol. The compiled literature work provided a clear impact of the biomass, microorganisms and catalysts to improve butanol production.

Chapter 3 - (peer-reviewed published paper) this study provides a benchmark for the intended bio-butanol production of this PhD study. This involved conducting a series of experiments to evaluate the use of *C. acetobutylicum* and *C. tetanomorphum* in ABE fermentation to convert sweet sorghum juice into bio-butanol. Notably, the ABE fermentation process was conducted in both single and co-culture fermentation. Even though the co-culture fermentation process yielded high acid concentrations with no solvents, which tends to divert from the main purpose of the study (Co-culture results presented in the Appendix).

Chapter 4 - (manuscript submitted to Biofuels) this study evaluated an alternative method which employs microwave heating over conventional heating for bio-ethanol to bio-butanol conversion. Experiments were conducted with three catalysts, i.e. magnesium oxide (MgO), platinum on alumina (Pt/Al_2O_3), and nickel on alumina (Ni/Al_2O_3). Ethanol conversion and butanol selectivity of each catalyst was qualitatively compared.

Chapter 5 - overall conclusions and recommendations are drawn from the generated results of the study.


1: General introduction

2: Literature review

3: Biochemical production of butanol: The aim of this section was to optimise the production of bio-butanol using different *Clostridium* species at different concentrations with hypothesis to increase butanol yield.

Link between Chapter 3 and 4: Due to low amounts of butanol obtained during fermentation caused by fermentation by-products and elongated fermentation time, an alternative route for butanol was developed for reduced time and enhanced butanol yield.

4: Chemical production of butanol: Here the aim was to develop a rapid microwave approach for butanol production using different catalysts with hypothesis to enhance butanol yield compared to the investigated ABE method.

Substrate: sweet sorghum juice extracted from the stalks was used for fermentation.

ABE fermentation: Different concentrations of *C. acetobutylicum* and *C. tetanomorphum* were used to produce butanol

Microwave catalytic reaction:
MgO, Pt/Al_2O_3 and Ni/Al_2O_3 were investigated for the production of butanol from bio-ethanol using 2^3 level factorial design. Ni/Al_2O_3 was further investigated with reaction time as a factor at constant temperature.

Substrate:
Ethanol derived from sugarcane molasses through fermentation was purchased from Rochelle Chemicals. The alcohol was purchased due to the cost implications of laboratory purification techniques for obtaining 99.9% purity as well as the amount of bio-ethanol volume which was required for the experiments. Please note, in our previous study (http://dx.doi.org/10.1016/j.biombioe.2014.09.022) bio-ethanol (containing water) was produced through a biological process. However, for the purpose of this study, a high grade bio-ethanol was required.

5: Overall conclusions

- From the findings of this study, it can be concluded that biomass as well as biomass derived ethanol have the potential to provide much more valuable products for the society
- The study also emphasized on the fast and convenient method (microwave technology) for future bio-butanol production when using Ni/Al_2O_3 as a catalyst. The microwave method is recommended over ABE and conventional heating methods.

Figure 1: Detailed scope for experimental chapters

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CHAPTER 2

n-Butanol derived from biochemical and chemical routes: A review

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Abstract

Traditionally, bio-butanol has been produced with the ABE (Acetone Butanol Ethanol) process using *Clostridium* species to ferment sugars from biomass. However, the route is associated with some disadvantages such as low butanol yield and by-product formation (acetone and ethanol). On the other hand, butanol can be directly produced from ethanol through aldol condensation over metal oxides/hydroxyapatite catalysts. This paper suggests that the chemical conversion route is more preferable than the ABE process, because the reaction proceeds more quickly compared to the fermentation route and fewer steps are required to get to the product.

Keywords: Bio-butanol, ABE fermentation, chemical synthesis

2.1 Introduction

Butanol (butyl alcohol) is a four-carbon alcohol that has been mainly used as a solvent, chemical intermediate, and extractant in cosmetics and pharmaceutical industries and also for the production of butyl acrylate and methacrylate (Dürre, 2007; Garćia *et al.*, 2011; Lee *et al.*, 2008). This class of alcohol is sometimes known as bio-butanol when produced biologically from the fermentation of starchy and sugar feedstock. Butanol mainly consists of four isomeric structures, i.e. *n*-butanol (n-C_4H_9OH), *sec*-butanol (sec-C_4H_9OH), *iso*-butanol (iso-C_4H_9OH) and *tert*-butanol (tert-C_4H_9OH) (Grana et al., 2010).

In recent years, *n*-butanol has caught the attention of researchers as an alternative biofuel to bio-ethanol. Although most researchers and industries previously focused on ethanol as a fuel than butanol (Hansen et al., 2005; Niven, 2005), butanol could be a better direct option. The straight chain alcohol (*n*-butanol) is considered as the next generation biofuel due to many advantages over ethanol (see Table 1), such as higher energy content, lower volatility, and it does not readily adsorb moisture (Dürre, 2007; Lee *et al.*, 2008; Nigam and Singh, 2011). The other added advantage is that the carbon atoms of butanol are double that of ethanol. It is known that the higher the number of carbon atoms, the more energy is contained (approximately 30% more energy) in butanol compared to ethanol (Durre, 2007; Warkett, 2008). The higher boiling point of butanol than that of ethanol causes butanol to take much longer to be burned in the motor engine than ethanol. It is also less corrosive and more suitable for distribution through existing petrol pipelines. The Reid vapour pressure of *n*-butanol is 7.5 times lower than that of ethanol, thereby making it less evaporative/explosive (Morone and Pandey, 2014). If used in a blend, *n*-butanol can be mixed in higher ratios than ethanol with petrol for use in existing cars without the need for modification, as the air-fuel ratio and energy content are closer to that of petrol (Sarathy et al., 2012; Campos-Fernandez et al., 2012). In addition, the high octane rating makes the alcohol more suitable to be used in internal combustion engines. A fuel with a lower octane rating is more prone to knocking (extremely rapid and spontaneous combustion by compression) and will lower efficiency. Knocking can also cause engine damage (Rudloff et al.,2013). Unlike other alcohols, the Environmental Energy Company

(US) confirmed that *n*-butanol can be used as a total replacement for petrol without any modifications to car engines (Brekke, 2007).

Table 1: Characteristics of butanol compared to ethanol (Gholizadeh,2009)

Characteristic	**Ethanol**	**Butanol**
Formula	C_2H_5OH	C_4H_9OH
Boiling point (^oC)	78	118
Energy density ($MJ.Kg^{-1}$)	26.9	33.1
Air fuel ratio	9.0	11.2
Research octane number	129	96
Motor octane number	102	78
Heat of vaporisation ($MJ.Kg^{-1}$)	0.92	0.43

Despite all the benefits associated with the use of the alcohol as a fuel, there are glitches associated with the bio-production of butanol, such as low production yield and high substrate cost. Many efforts are being directed towards the utilisation of lower-cost substrates such as maize stover (He and Chen, 2013) agricultural waste (Cheng et al., 2012) and rice straw (Ranjan et al., 2013), barley straw (Qureshi et al., 2013) and switchgrass (Jain et al., 2014). First-generation bio-butanol causes problems, ranging from a negative impact on food security, increased food prices and net energy losses (Martin, 2010). Other studies have focused on the utilisation of crude glycerol that is obtained as a byproduct during biodiesel production to lower the impact of biofuel production on food security (He and Chen, 2013; Khanna et al., 2013).

The search for cheaper second-generation substrates, technology and other carbon sources will be required if renewable biofuels are to make more significant advantages into the world’s energy portfolio. Lignocellulose is potentially an alternative feedstock for bio-butanol production and therefore a more efficient bioconversion method of cellulose and hemicellulose is required for the economic success of the industrial production of bio-butanol (Howard et al., 2003; Kumar et al., 2009). Research into more effective conversion of biomass material using economically feasible processes is therefore of principal importance. The use of chemical methods for direct conversion of ethanol to butanol is also reported.This study gives a detailed review of the current production options for *n*-butanol from biomass using biochemical and chemical strategies. In addition, the review discusses the history of butanol, various synthesis mechanisms, their advantages and drawbacks and also the future in the current bio-butanol industry on the global scenario.

2.2 History of bio-butanol production from biomass

Acetone butanol ethanol fermentation was discovered by a French microbiologist, Louis Pasteur, in 1861. Research in butanol came to life again as large quantities of acetone were demanded in World War I when Fernbach and Strange (1912), filed a patent for the process, which was tested in the United Kingdom (UK). However, there was a need for an organism that could increase acetone production. Strange and Chaim Weizmann succeeded in isolating *Clostridium acetobutylicumin* from a garden soil for ABE fermentation that could produce large amounts of acetone (Weismann,1922).

During World War I, it was important to produce large quantities of acetone since in Britain it was used as an essential chemical for the production of cordite that was used as an alternative to gunpowder. With the increasing demand for butanol after the war, the first large-scale industrial plants were created in Canada and the USA. After 1936, a number of ABE production industries were established in the Soviet Union, Japan, China, South Africa and Egypt. By 1945, during the start of the Second World War, Japan commenced the production of butanol from sugar plants mainly as fuel for airplanes (Ezeji et al., 2010).

In the 1950s, a petrochemical route for *n*-butanol production emerged. The process was mostly based on the aldol condensation of acetaldehydes, followed later by dehydration and then the hydrogenation of crotonaldehyde. With this fast-growing industrial discovery, also known as Oxo synthesis, the fermentation processes were abandoned (Uyttebroek et al., 2015). For example, by the 1960s, most of the industrial ABE fermentation facilities were closed due to cheap oil prices that favoured the chemical production route. The last factory was closed in 1986 in South Africa. The chemical route did not last for a long time until the rise in crude oil prices when industrial ABE fermentation facilities started to emerge again in China and Brazil. To date, many plants have been established in several locations globally, including the USA, Slovakia, France and UK where bio-butanol is produced for use as a fuel.

The *n*-butanol (as biofuel) application was demonstrated by running an old Buick on pure *n*-butanol in the year 2005. The reported fuel consumption increase was 9% higher than traditional petrol (Durre, 2008). Regardless of the increase, emissions of carbon monoxide (CO), hydrocarbons and nitrogen oxides (NOx) were substantially reduced, due to less negative characteristic effects (Table 1) that butanol has on the environment. This trend is regarded as a positive impact towards the environment. This resulted in the Fuel Butyl Company to increase the capacity to produce 10 L of *n*-butanol per 25 kg of maize. A year after 2005, two major global players, BP and DuPont, also announced plans for manufacturing plants to produce *n*-butanol through fermentation processes. The first new commercial plant was built by BP in Saltend (UK) with a capacity to produce 420 million L of *n*-butanol (per annum) through fermentation (Durre, 2008; Warkett, 2008).

2.3 Bio-chemical production route

The most attractive routes for the production of bio-butanol are through the fermentation of sugar (Chen et al., 2014), glycerol (Yadav et al., 2014) or lignocellulose (Yang et al., 2014) feedstock in the presence of different micro-organisms from the Clostridiaceae family. The ABE fermentation process has many benefits, since it largely depends on the availability of inexpensive and abundant raw materials. Bio-butanol fermentation from biodiesel derived glycerol is also considered as an alternative route, since it uses waste products from biodiesel production. The renewed interest of butanol production has not only highlighted its use as a chemical, but also as an alternative biofuel, due to a rise in oil prices (Lee et al., 2008; Kumar and Gayen, 2011).

2.3.1 Metabolic pathway-ABE fermentation

The metabolic pathway utilises glucose derived from the hydrolysis of carbohydrates (Figure 1, step 1) that are broken down by amylase enzyme to form monosaccharides, and solvents by *C. acetobutylicum* via anaerobic fermentation (Ezeji et al., 2010; Huang et al., 2010). The mechanism of ABE has been elucidated, carbon from carbohydrates in the form of pentose, and hexose sugars (mono-, di- tri-, and polysaccharides) are metabolised via the Embden-Meyerhof pathway (Step 2) to pyruvate. The degradation of 1 mole of sugar leads up to 2 moles pyruvate with a net formation of 2 moles of adenosine triphosphate (ATP) and 2 moles of nicotinamide adenine dinucleotide (NADH).

Pyruvate is further converted to acetyl-CoA and carbon-dioxide (CO_2) (Figure 1, step 3). Thereafter, step 4 shows acetyl CoA being converted to other intermediates (acetaldehyde, butyraldehyde) that ultimately lead to oxidised products (acetone, acetate), and reduced products such as butanol and ethanol. The first occurrence of intermediates and acid formation is known as acidogenesis, and this occurs under specific growth conditions, such as pH values >5 and iron limitations. Therefore, ATP is continuously generated during this process (Nigam and Singh, 2011).

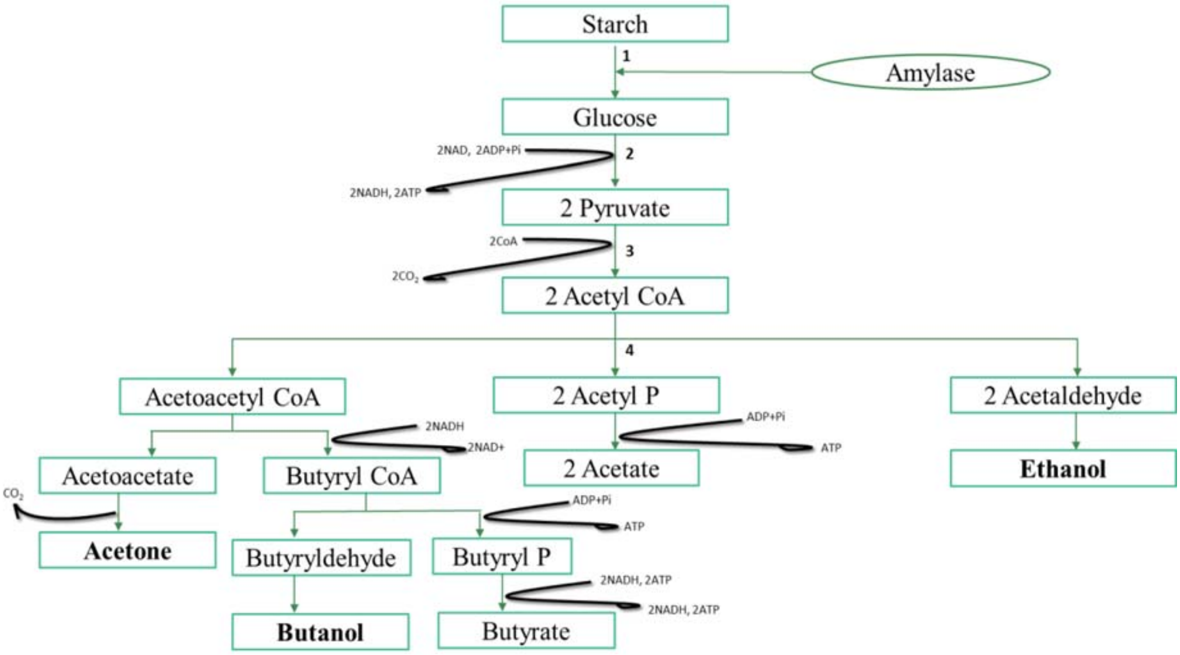

Figure 1: Biochemical pathway of ABE fermentation (Durre, 2008)

During the accumulations of organic acids (Acidogenesis), the culture pH reduces due to the metabolic switch of *C. acetobutylicum* from acidogenesis to solventogenesis (Durre, 2007; Durre, 2008). Organic acids are utilised for the formation of solvents (Solventogenesis). The second phase of ABE fermentation is solventogenesis, a stage during which acids are re-assimilated to produce acetone, butanol, ethanol, acetic acid, butyric acid, hydrogen, and carbon dioxide as the main products. This stage of fermentation occurs frequently in various bacterial strains of Clostridia utilising a wide range of carbon sources such as starch. An abundant and cheaper feedstock investigated and identified as alternative substrates for butanol production via ABE fermentation is lignocellulosic biomass, which includes agricultural residues, namely maize fiber, wheat straw and switchgrass (Ezeji and Qureshi, 2007; Huang et al., 2010; Qureshi et al., 2010).

Acetate and butyrate are assimilated to their corresponding CoA derivatives catalysed by the acetoacetyl-CoA:acyl-CoA transferase, with acetoacetyl-CoA as the CoA donor. The utilisation of acetate and butyrate occurs via the acetoacetyl-CoA:acetate/butyrate:CoA transferase (CoAT) pathway with the formation of acetone. Butyryl-CoA converts to butaraldehydes, and finally to ethanol and butanol. Meanwhile, acotoacetyl-CoA converts to acetone and acetaldehydes to ethanol (Jones and Woods,1986; Huang et al., 2010; Jin et al., 2011).

2.3.2 Typical feedstock for butanol production

Bio-butanol is usually produced from food crops, which are considered first-generation feedstock; nevertheless, second-generation feedstock that is not considered for human consumption is preferred. Second-generation feedstock are desired in ABE fermentation because they are non-food and does not utilise feedstock which can serve as food. Therefore, processing of second generation feedstock can make use of a greater proportion of a plant material and is considered to have long-term economic advantages. Research is also currently in progress for third-generation feedstock, however few literature studies have already been established.

Bio-butanol can be produced through a variety of different micro-organisms from the Clostridiaceae bacterial class. Clostridia are well-known to produce several products, which cannot be achieved through the use of chemical synthesis (Ezeji et al., 2004). Clostridia are rod-shaped, spore-forming, gram-positive bacteria and very strict anaerobes. The yield of butanol varies, depending on the type of biomass and bacteria used, and different parameters are relevant. The forthcoming section is aimed at illustrating literature findings related to butanol production using different *Clostridium* species and biomass thereof.

2.3.2.1 First-generation bio-butanol

First-generation bio-butanol requires a relatively simple process to be produced, mostly afforded by the fermentation of mostly hexose sugars. These sugars are derived from the hydrolysis of starch-rich crops such as maize, wheat, rice and cassava. Prior to use, the raw materials (grains) are usually hydrolysed into dextrose, which can be subsequently bio-converted into glucose using glucoamylase enzymes. Several researchers have demonstrated that first-generation butanol production can be achieved at significantly high yields. Table 2 shows the literature associated with different types of feedstock for first-generation bio-butanol, the various micro-organisms used and their product yields.

Table 2: Examples of first generation biomass used in butanol fermentation

First-generation feedstock	**Fermentation conditions (T°C and pH)**	***Clostridium* species**	**Yields**		**Productivity (g/L)**	**Product distribution**	**Reference**
			g/L	**g/g**			
Cassava starch	37 and 5	*C.beijerincki* *tyrobutyricum*	6.66	0.18	0.96	Butanol	(Lin et al., 2013)
Glucose	37 and over 4	*C.acetobutylicum* CICC 8012			0.13	Butanol	(Chen et al., 2014)
Cassava flour	37 and pH controlled	*C.acetobutylicum* DP 217	574.3		0.76	ABE	(Li et al., 2014)
Oil palm sap	37 and 6	*C.acetobutylicum* DSM 1731	14.4	0.35		Butanol	(Komonkiat and Cheirsilp, 2013)
Maize meal	37 and 6	*C. beijerinckii*, BA101	26			Acetone and Butanol	(Ezeji et al., 2004)

Lin et al. (2013), investigated co-cultures of *Clostridium beijerinckii* and *Clostridium tyrobutyricum* to enhance butanol production yield. The experiments were conducted using glucose, cassava starch, or cane molasses. A fibrous-bed bioreactor (FBB) was used for immobilised fermentation. According to the findings of the study, two-strain co-culture butanol production yields and volumetric productivity increased compared to using a single culture. The reported butanol production was 6.66 g/L, yield and productivity 0.18 $g.g^{-1}$ and 0.96 g/L/h, respectively, when using cassava as a starting material. An acetone-butanol-ethanol (ABE) yield of 0.36 g/g was produced. The study confirms that co-culture fermentation could enhance butanol productivity.

According to Chen et al. (2014), glucose as a starting material produced 0.14 and 0.13 $g.\ L^{-1}.\ h^{-1}$ butanol using *Clostridium acetobutylicum* strain CICC 8012. Fermentation was monitored under slight pressure on a continuous and closed-circulating fermentation (CCCF) system. Fermentation conditions were 37^{o}C, and the pH value was adjusted to over 4. As fermentation proceeded, glucose concentration was maintained at approximately 30 $g.\ L^{-1}$ by adding it every two hrs of fermentation. The study indicated that pressures have no effect on the *Clostridium acetobutylicum* strain CICC 8012's performance during fermentation.

The production of acetone-butanol-ethanol (ABE) from cassava was investigated by (Li et al., 2014). Fermentation was done using a pervaporation (PV)-coupled process. ABE products were *in situ* removed from the fermentation broth to alleviate the toxicity of the solvent to the *Clostridium acetobutylicum DP217*. Compared to the batch fermentation without PV, the glucose consumption rate and solvent productivity increased by 15% and 21%, respectively, in the batch fermentation-PV-coupled process, while in continuous fermentation-PV-coupled processes running for 304 h, the substrate consumption rate, solvent productivity and yield increased by 58%, 81% and 15%, reaching 2.02 g/L, 0.76 g/L/h, and 0.38 g/g, respectively. After phase separation, a final product containing 574.3 g/L ABE with 501.1 g/L butanol was obtained. Therefore, the results from the study proved that the fermentation-PV-coupled process has the potential to decrease the cost in ABE production.

Kominkiat and Cheirsilp (2013), conducted a study using a sap of palm oil to produce bio-butanol. The findings gave an indication that palm oil could be used as a feedstock as it was effective for high butanol yield without any nutrient supplementation. An amount of 14.4 g/L butanol was obtained from the sap, which contained a sugar concentration of 50 g/L with a butanol yield of 0.35 g/g.

Ezeji et al. (2004), tested liquefied maize meal for acetone-butanol production. Fermentation was done using hyper-amylolytic *C. beijerinckii* BA101. *C. beijerinckii* has the ability to utilise starch and accumulate higher butanol concentrations of 17–21 g/L in the medium. Batch fermentation was used and a solvent (acetone and butanol) recovery of 26 g/L was obtained. The results show that maize meal is an effective starch biomass to yield butanol using a batch scale in the presence of *C. beijerinckii* BA101.

2.3.2.2 Second-generation bio-butanol

Biofuels from different agricultural residues and part of the plant biomass are often termed second-generation because the fuels are derived from feedstock that is non-edible residues of food crop production or non-edible plant biomass (e.g. grasses, trees and energy crops). The main advantage of the production of second-generation biofuels is that there is no competition with the food-feed chain and their availability is diverse. Lignocellulosic materials are associated with low cost, sufficient abundance and usually they generate low net greenhouse emissions, and therefore must be ideal precursors to produce biofuels. Second-generation liquid biofuels are generally produced by two fundamentally different approaches, i.e. biological or thermochemical processing due to their structural complexity. Table 3 reveals literature on some of different second-generation biomass that are currently used to produce butanol, and the corresponding *Clostridium* bacterial strains.

Table 3: Examples of second generation biomass used in butanol fermentation

Second-generation biomass	Fermentation conditions (T°C and pH)	*Clostridium* species	Yields		Productivity (g/L/h)	Product distribution	References
			g/L	g/g			
Barley liquor silage	37 and 6.5	*C. acetobutylicum* DSM 1731	9.0			ABE	(Yang et al., 2014)
Glycerol	37 and over 6.5	*C. acetobutylicum* KF 158795	13.57			Butanol	(Yadav et al., 2014)
Rice straw	37 and 6.7	*C. sporogenes* BE01	5.52			Butanol	(Gottumukkala et al., 2013)
Oil palm trunk fiber	37 and 6	*C. beijerinckii* TISTR 1461	10	0.41		Butanol	(Komonkiat and Cheirsilp, 2013)
Crude cellulose	37 and 6.7	*C. acetobutylicum* DP 217		0.33		Butanol	(Tippkotter et al., 2014)
Spoilage palm fruits	30 and 6	*C.acetobutylicumATCC824 and B. subtilis DSM 4451*	21.56	0.42	0.30	ABE	(Abd-Alla and El-Enany, 2012)

Several research attempts have been developed to utilise second-generation biomass with appropriate fermenting bacteria to yield higher butanol. Studies developed a *C. acetobutylicum* strain that can directly utilise cellulose as feedstock (Lee et al., 2008; Jin et al., 2011). There is evidence that *C. acetobutylicum* ATCC 824 contains a cellulosome, that is, a cellulose-degrading multi-enzyme complex consisting of several catalytic components surrounding a scaffold protein. In an effort to make *C. acetobutylicum* utilise cellulose directly, the cellulase gene from *C. cellulovorans* or the gene encoding the scaffold protein from *C. cellulolyticum* and *C. thermocellum* was introduced into *C. acetobutylicum* (Jin et al., 2011).

Although the gene has been identified, more studies still need to be done for the characterisation of the existing cellulase gene cluster in *C. acetobutylicum* before further metabolic engineering. As a result, this will further increase the feasibility of lignocellulosic material usage compared to first-generation biofuels. The production of second-generation biofuel requires more sophisticated processing production equipment, more investment per unit of production and larger-scale facilities. To achieve the potential energy and economic outcome of second-generation biofuels, further research, development and application are required on feedstock production and conversion technologies. The future production of ethanol is expected to include both the use of traditional grain/sugar crops and lignocellulosic biomass feedstock (Yadav et al., 2014).

Various studies have been conducted using different second-generation feedstock. Batch fermentations of barley silage liquor were supplemented with gelatinised barley grain, and the results produced good fermentability butanol yields of 0.20, 0.17 and ABE yields of 0.28, 0.26 $g.g^{-1}$ monosaccharide (Yang et al., 2014). The study showed that starch in silage could be a possible replacement of media components that provide the nutrients for butanol fermentation.

Trunk fiber was utilised as one of the second-generation feedstock types (Komonkiat and Cheirsilp, 2013). The feedstock was firstly hydrolysed to fermentable sugars prior to fermentation. A number of Clostridial strains were screened, and *Clostridium beijerinckii* TISTR was the most suitable organism for utilising sugars from hydrolysed trunk fiber with the highest amount of 10 g/L butanol concentration and butanol yield of 0.41 g/g. The results presented herein suggest that oil palm trunk is a promising renewable substrate for bio-butanol. A study conducted by Cai et al. (2013), showed that sweet sorghum bagasse (SSB) hydrolysate produced 12.3 ± 0.1 g/L butanol. The results were only obtained after the pervaporation membrane was used for sweet sorghum bagasse detoxification. This was done to remove all the toxic substances which usually hinder the fermentation process. Lignin is one of the major inhibition components. Although more inhibitors are often found during fermentation stage. Detoxification (pretretment) of raw material prior to fermentation is pivotal for obtaining improved butanol concentration. Such fermentation inhibitors include high concentration of sugars which leads to high amounts of acetic acid, and this consequently supresses bacterial cell growth for fermentation.

Gottumukkala et al. (2013), used an acid hydrolysed rice straw for bio-butanol production. *Clostridium sporogenes* BE01 was used as a fermenting bacterium. This strain resulted in butanol yield of 3.43 g/L and a total solvent yield of 5.32 g/L. After the conditions of ABE fermentation were enhanced, bio-butanol reached 5.52 g/L. The study indicated the potential of rice straw to be utilised as biomass for bio-butanol production.

Yadav et al. (2014), used glycerol as feedstock for bio-butanol production. Twenty anaerobic bacteria were screened for biomass conversion. Among the 20, *Clostridium acetobutylicum* KF158795 was found to be the most effective bacterium in producing bio-butanol. Results showed that the strain was able to utilise glycerol as a sole carbon source to produce 1.4 g/L of butanol. After optimisation, production increased to 13.57 g/L of butanol. Tippkotter et al. (2014), also conducted research on crude cellulose as a starting material for bio-butanol production using *Clostridium acetobutylicum*. The finding was 0.33 g/g butanol yield. Various researchers have shown the use of *Clostridium acetobutylicum* as a suitable strain for bio-butanol production, and the study also supports the literature.

Spoilage date palm fruits were used as substrate for ABE production through a mixed culture of *Clostridium acetobutylicum* ATCC 824 and *Bacillus subtilis* DSM 4451. The total

ABE production of 21.56 g/L was achieved from the date fruits. The productivity and yield obtained were 0.30 g/L/h and 0.42 g/g, respectively (Abd-Alla and El-Enany, 2012).

Marchal et al. (1992), used maize cobs to produce an ABE yield of 0.96 g/L/h in a 48 m^3 reactor. Fed-batch and continuous fermentation techniques have been developed in addition to conventional batch fermentation to utilise concentrated substrates and eliminate downtime, thereby reducing the reactor size and capital cost with enhanced reactor productivity. Cell immobilisation with bone char, brick and cotton towels as supporting materials has also been applied in ABE fermentation to achieve high cell density and reactor productivity.

Khanna et al. (2013), used biodiesel-derived crude glycerol as a feedstock to produce *n*-butanol with a maximum yield of 5 g/L/h butanol via an anaerobic fermentation pathway, using immobilised *Clostridium pasteurianum* cells. This was the first report to apply immobilised cells to convert glycerol to butanol. A mutant strain of *C. pasteurianum* that can tolerate high concentrations of crude glycerol was developed and reported a maximum glycerol utilisation rate of 7.59 g/L/h and a butanol productivity of 1.80 g/L/h.

With the detailed literature reports on first and second-generation bio-butanol and stringent food regulations, there are still drawbacks associated with the feedstock. This is ascribed to the processing stage and storing of second-generation during harvest season. However, there is another promising alternative such as third-generation biomass for bio-butanol production, which can be produced in large quantities with less processing compared to lignocellulose biomass.

2.3.2.3 Third-generation bio-butanol

Algae have increasingly became one of the promising feedstock arising from its vast availability (Yeong et al., 2018). It is catagorised as a third-generation feedstock. A few reports exist on bio-butanol production from algae. The reason for this is that most species have a high oil content of approximately 50% (Ullar et al., 2015), and that also makes it suitable for biodiesel production. The cell residue that is left after oil extraction can be further used for the production of bio-butanol. The two distinct types of algae are microalgae and macroalgae. Microalgae are made up of unicellular organisms that are classified as microscopic. The latter contains multiple cells with a structure like a plant with roots, stems and leaves. They are categorised into i.e. red, green and brown, depending on their pigmentation. The specific characteristics of macroalgae are lower protein and lipid content, but higher carbohydrates content compared to microalgae (Monlau et al., 2014). Most research reports (Van der Wal et al., 2013; Maity et al., 2014; Castro et al., 2015; Cheng et al., 2015) have mainly focused on microalgae over macroalgae, e.g. seaweed (Potts et al., 2012; Ullar et al., 2015).

Table 4: Examples of third-generation biomass used in butanol fermentation

Third-generation biomass	**Fermentation conditions (T°C and pH)**	***Clostridium* species**	**Yields**		**Productivity (g/L/h)**	**Product distribution**	**References**
			g/L	**g/L**			
Mixed microalgae	35 and 6.5	*C. Saccharoperbutylacetonicum* N1-4	3.74			Butanol	(Castro et al., 2015)
Wastewater algae	35 and over 6.5	*C. Saccharoperbutylacetonicum* N1-4	9.74	0.311	0.102	ABE	(Ellis et al., 2012)
Micro algae biodiesel residues	37 and 6	*C. acetobutylicum* ATCC 824		0.13		Butanol	(Cheng et al., 2015)
Green seaweed (*Ulva lactuca)*	37 and 6.0-6.4	*C. beijerinckii* NCIMB 8052		0.35		ABE	(Van der Wal et al., 2013)
Macroalgae	37 and 6	*C. beijerinckii* ATCC 35702	4			Butanol	Potts et al., 2012)

Castro et al. (2015), first optimised acid hydrolysis of mixed microalgae for sugar release. The sugars were subsequently fermented to produce ABE by *Clostridium saccharoperbutylacetonicum* N1-4. The findings provided an optimal sugar yield of 166.1 g per kg of dry algae, with concentrations of 3.74 g/L butanol. Ellis et al. (2012), also conducted acetone, butanol and ethanol (ABE) fermentation by *C. saccharoperbutylacetonicum* N1-4 using wastewater algae. The higher ABE yield of 0.311 g/g and volumetric productivity of 0.102 g/L/h were obtained when enzyme supplementation on the biomass was done. It was concluded that the use of wastewater algae to produce industrial solvents that are of high value could have substantial implications in terms of economic use. The study showed that the use of organic waste is an alternative method of utilising approximately 100% of the biomass. Additionally, Cheng et al. (2015), conducted research on the use of microalgae biodiesel residues using *C. acetobutylicum* that produced 3.86 g/L of butanol with a yield of 0.13 g/g carbohydrate during ABE fermentation. This observation confirmed that bio-butanol production from microalgae biodiesel residues is attainable. However, advance research on fermentation approaches is required to improve bio-butanol yield.

Van der Wal et al. (2013), conducted research on the application of potential green seaweed *Ulva lactuca* for the production of acetone,butanol and ethanol. Two *Clostridium* species were used, i.e. *C. acetobutylicum* and *C. beijerinckii*. According to the findings, *C. beijerinckii* was the organism capable of producing ABE of 0.35 g/g while *C. acetobutylicum* produced mainly organic acids (acetic and butyric acids). These results demonstrate the great potential use of *U. lactuca* as feedstock for fermentation. On the other hand, Potts et al. (2012), reported potential feedstocks to produce bio-butanol from a macroalgae. They found that it can produce approximately 4 g/L butanol by *C. beijerinckii* and *C. saccharoperbutylacetonicum*, with *C. beijerinckii* being the highest butanol-producing organism.

The topic of algal conversion to bio-butanol is not new, although thorough research has not been done on the different algal feedstock. Table 4 ascertains the different feedstock (microalgae and macroalgae) for bio-butanol production, conditions used, and the amounts produced thereof.

This section reveals a number of studies that have been conducted on different biomass and *Clostridium* species for bio-butanol production. The results reported show different bio-butanol yields. Therefore, the effect of diversity on bio-butanol production has been shown. However, there are still setbacks in technology discoveries, and inhibitions on optimisation of the existing bio-butanol production process (fermentation).

2.3.2.4 Inhibitions and other problems associated with ABE fermentation

Although a number of feedstock and microorganisms have successfully been utilised to produce bio-butanol, there are numerous drawbacks associated with ABE fermentation that hinder its economic viability compared to petrochemical synthesis. Some of the identified limitations of the ABE based on the process aspect are as follows:

1. Low final butanol concentration (<20 g/L) caused by inhibition during fermentation

2. Low yield of butanol due to hetero-fermentation (0.28-0.33 g/g)

3. High cost of butanol recovery from low-concentration yields (Jin et al., 2011).

Not only is inhibition a major challenge for the ABE fermentation, research has also shown that optimization of biomass processing is challenging. Current ABE fermentation depends on dual purpose biomass which is used for food consumption as well as ABE production. However, production costs of ABE are very high, therefore efforts must be directed towards decreasing feedstcock processing costs. Rational approaches and advanced knowledge is necessary for the effective strategies to limit the inhibitors while improving the yield of butanol are needed. This can be archieved by advancing ABE technology or studying chemical production of *n*-butanol using numerous catalysts.

2.4 Chemical synthesis of *n*-butanol from ethanol

As the interests in bio-butanol keep on growing, there is also a rising attention on the use of chemical routes to produce *n*-butanol. Since the use of fermentation still faces significant technical and economic challenges, such as finding efficient microorganisms to convert fermentable sugars to bio-butanol, it is still mandatory to find other alternate routes. On the other hand, the chemical route usually involves a simple step in the presence of catalysts that are employed to achieve suitable ethanol conversion into *n*-butanol at relatively higher yield and conversions. The primary advantage of the chemical route is that only one step is required to produce *n*-butanol from ethanol, whereas the bio-chemical route may involve several steps. Chemical processes for *n*-butanol production are wide-spread and have been well documented (Ogo et al., 2011; Uyttebroek et al., 2015).

2.4.1 Reaction mechanism of *n*-butanol formation from ethanol

There are three consecutive reactions associated with the conversion of *n*-butanol from ethanol. The first liquid phase reaction is the dehydrogenation of ethanol to form acetaldehyde (Santacesaria et al., 2012), followed by aldol condensation of acetaldehyde (Juben et al., 2007) and then hydrogenation to *n*-butanol (see Figure 2). Ndou et al. (2003) and Ueda et al. (1992) proposed a mechanism for the gas phase reaction of ethanol over a zeolite catalyst to synthesise butanol. According to Uyttebroek et al. (2015), the gas phase reaction does not involve the formation of intermediates such as acetaldehydes and crotonaldehydes. To date, few reports have been made on the gas phase reaction formation of *n*-butanol and the reaction mechanism. Ethanol to *n*-butanol conversion is an important industrial process that has been used to increase the carbon number of alcohols by coupling two molecules. Aldol condensation was first studied by Marcel Guerbet in the 1890s, and it is widely known as the Guebert reaction (Uyttebroek et al., 2015). Although this method is rarely utilised nowadays, it may again become significant in the future.

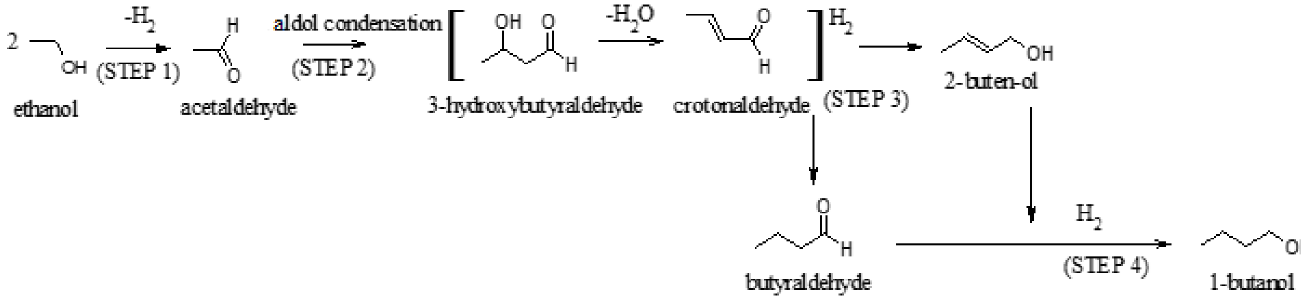

Figure 2: The reaction mechanism for production of *n*-butanol from ethanol in a liquid phase (Ogo et al., 2011).

2.4.1.1 Dehydrogenation

Dehydrogenation of ethanol allows the removal of hydrogen in a chemical reaction. In this instance, ethanol is mostly used to synthesise ethyl acetate and acetaldehydes. Santacesaria et al. (2012), demonstrated that dehydrogenation is of outmost importance in the industrial process to produce fine chemicals, petrochemicals and oleochemicals. In Scheme 1, the first step (labelled as step 1) presents the dehydrogenation of a molecule followed by aldol condensation in the presence heterogeneous catalysts to produce the desired product (Fujita et al., 2001).

2.4.1.2 Aldol condensation

Juben et al. (2007), described aldol condensation as a well-known phenomenon used to produce a β-hydroxyaldehyde or β-hydroxyketone. This occurs in an organic reaction containing enol or an enolate ion that reacts with a carbonyl compound and followed by dehydration to give a desired product. Aldol condensation is one of the important organic synthesis methods with the advantage to form carbon-carbon bonds. The Guerbet reaction is one of the aldol condensation types whereby an *in-situ* aldehyde is formed from an alcohol and then self-condenses to the dimerised alcohol (Casale et al., 2007).

2.4.1.3 Hydrogenation

The subsequent step after aldol-condensation is hydrogenation of aldol-adducts to increase their solubility in the aqueous phase. In addition, selective hydrogenation of the furan ring in HMF and furfural can lead to additional carbonyl-containing compounds that can undergo aldol self-condensation to form heavier alkanes. Thermodynamic considerations favour hydrogenation of the C=C bond over the C=O bond for hydrogenation reactions involving unsaturated aldehydes Reaction kinetics considerations also favour the hydrogenation of the C=C bond over the C=O bond for small molecules, whereas steric constraints for larger molecules decrease the rates for hydrogenation of C=C bonds. Accordingly, the C=C bonds of furfural are less reactive than the C=O bond, probably due to steric effects, making the production of tetrahydrofurfural (THF2A) by hydrogenation of furfural difficult (Juben et al., 2007). The chemical approach is improving through ongoing research to develop an appropriate catalyst, or pair of catalysts, for the hydroformylation of alkenes to form aldehydes and the subsequent hydrogenation of the aldehydes to produce alcohols (Zakzeski et al., 2012).

2.4.2 Catalysts used for *n*-butanol synthesis

Several researchers, to name few (Carvalho et al., 2012; Dowson et al., 2013; Freitas et al., 2014) have devoted their work in a quest to discover suitable catalysts that would be able to produce high yields of *n*-butanol (see Table 5). These new technologies are compared to that of existing biological technologies in order to understand the two approaches. Table 5 presents several catalysts that have been previously used to produce *n*-butanol with ethanol as a feedstock.

Table 5: Examples of few catalysts and conditions studied in the literature

Catalysts	**Catalyst loading**		**Catalytic test temperature (°C)**	***n*-Butanol selectivity (%)**	**References**
	mg	**wt%**			
Mg- and Al-mixed oxides	300		500	>20	(Carvalho et al., 2012)
ZrO_2-supported Cu	500		300	≥10	(Freitas et al., 2014)
Ni/Al_2O_3	10-50		250	80	(Riittonen et al., 2012)
Hydroxyapatite			350-450	50	(Scalbert et al., 2014)
Na/ZrO_2		1	340-400	-	(Kozlowski and Davis, 2013)
Ni/ γ-alumina	3000-3500		250	62	Ghaziaskar and Xu, 2013)
Co^{2+} / Ca^{2+} hydroxyapatite		1.35	120-240	-	(Elkabouss et al., 2004)
MgO	500		450	-	(Juben et al., 2007)

A series of literature work has been conducted on the direct catalytic conversion of ethanol to *n*-butanol. The observed findings differed depending on the reaction conditions and the catalyst used. Freitas et al. (2014), evaluated copper (Cu) supported onto zircornia catalysts with different Cu content (5-30 wt%) for butanol production. Their observations showed ethanol conversion of 33.3% and selectivity as low as 16.6% with Cu content (10 wt%). Carvalho et al. (2012), studied Mg- and Al-mixed oxides for the conversion of ethanol to *n*-butanol. The results proved that Mg and Al havea potential in achieving selectivity of 20%. However, the findings were not as promising as the other studies conducted, where Riittonen et al. (2012), studied direct catalytic one-pot valorisation of bio-ethanol to 1-butanol over different alumina supported catalyst and found 80% *n*-butanol selectivity.

Scalbert et al. (2014), investigated a commercial hydroxyapatite catalyst and produced *n*-butanol selectivity of 50%. Kozlowski and Davis (2013), studied the effect of depositing sodium to zirconia (Na/ZrO_2) on the acid-base surface properties in the catalytic conversion reaction of ethanol. Their findings showed that overall selectivity of ethanol to butanol was improved when catalyst of 1.0 wt% Na/ZrO_2 was used. Ghaziaskar and Xu (2013) carried out a promising one step continuous-flow catalytic conversion of ethanol to butanol. Their reaction conditions showed a butanol selectivity of 62% in the presence of Ni/γ-Al_2O_3 catalyst. Ndou et al. (2003), studied different catalysts including BaO, CaO, MgO and γ-Al_2O_3 derivatives for conversion of ethanol into 1-butanol. The catalytic conversion showed highest selectivity of butanol over MgO in comparison to other catalysts. Hydroxyapatite are among the promising catalysts showing high selectivity towards *n*-butanol. They suffer from deactivation during conversion reactions by high molecular weight by-products. Elkabouss et al. (2004), prepared a series of exchanged cobalt/calcium hydroxyapatite for catalytic reactions. The introduction of cobalt into hydroxyapatite matrix significantly improved the catalytic activity.

Leon et al. (2011), showed that magnesium (Mg)- and aluminum (Al)-mixed oxide catalysts with a high sensitivity of strong basic sites are the most selective to *n*-butanol. The same authors also observed a very high selectivity to ethylene, which is associated with the presence of acid sites on the surface of these mixed oxides. Marcu et al. (2009), also verified that the selectivity to *n*-butanol increases when the density of strong basic sites increases as was observed when Cu-Mg-Al-based catalysts were used. It was concluded that the formation of *n*-butanol requires both basic and acid sites. According to some researchers (Carvalho et al., 2012; Leon et al., 2011), a specific surface atomic arrangement composed of two acid sites and one basic site is needed in order to adsorb the acetaldehyde molecules during the aldol condensation step.

The use of various heterogeneous catalysts for ethanol conversion to *n*-butanol has been extensively researched by the aforementioned studies. However, the studies are limited to the evaluation of catalyst performance, and they give less information on the use of these catalysts at a commercial scale. In developing countries such as South Africa, butanol production has not been well documented. It is necessary to evaluate all the possible technologies that can aid in fuel production to boost the country's economy. This will also allow the comparison of different processes and their impacts on the entire production process, which would be much harder to achieve in an experimental scale.

2.5 Microwave Catalysis

A reduction in reaction time, lower energy consumption and improved product yield and selectivity are some of the advantages in using microwave technology to promote chemical reactions (Eslami et al., 2017). Many studies have evaluated the use of microwave heating (see Table 6) as compared to conventional heating during chemical reactions (Safari et al., 2014; Baran and Mentes, 2016; Mirzaei and Neri, 2016). It has been shown that microwave heating affects a large increase in reaction rate compared to conventional heating. Microwave energy only heats the desired material with less/no harmful greenhouse gas emissions from the heat source.

Among the application of catalytic reaction for development of sustainable organic processes, catalytic carbonylation reaction and hydroformylation must be highlighted since they are commonly used for the production of many chemicals and gases (Bowman et al., 2008; Pineiro et al., 2017). An example of microwave-assisted catalytic reactions is a study conducted by Granados-Reyes et al. (2018) on the transesterification of glycerol towards glycerol carbonate. Lestinsky et al. (2017) also utilised a microwave for pyrolysis on spruce sawdust in the presence of catalysts to maximize the yield of hydrogen. Another study focused on the upgrading of Jatropha oil biodiesel using a microwave (Wei et al., 2018). However, more literature is currently focusing on microwave catalyst synthesis for enhancing catalyst activity (Li et al., 2016; Mnatsakanyan et al., 2016; Wang et al., 2017; Gangurde et al., 2018) and not on the formation of different alcohols such as butanol.

Table 5: Examples of some of the catalysts and microwave conditions studied in the literature

Catalysts	**Methods**	**Time (hrs)**	**Microwave conditions**	**Products**	**References**
[HSO_4-BMIM]HSO_4	Transesterification	6.43	168 W	98.93% biodiesel	Ding et al., 2018
Ag-Rh nanoparticles	Dehydrogenation of cychlohexane	2	300°C	400mmol/g_{met}/min of H_2	Pande et al., 2018
$BaCl_2$-TiO_2-SnO_2	Dehydrogenation of ethane	1	650°C	23.9% Ethylene	Bolotov et al., 2018
Cr/ZrO_2	Dehydrogenation of propane	5	550°C	Propene	Oliveira et al., 2018
CaO	Transesterification of palm oil	0.06	900W	96.7% Fatty acid methyl esters	Khemthong et al., 2012

In a paper by Ding et al. (2018), three acidic imidazolium ionic liquids were successfully synthesized and tested for their activity towards the transesterification reaction for the formation of biodiesel under 168 W microwave irradiation condition. Among the three tested ionic liquids, $[HSO_3\text{-}BMIM]HSO_4$ was proved to be the most suitable catalyst for the production of biodiesel. In summary, the study showed that it could provide an environmentally friendly and efficient method for the preparation of biodiesel, while the combination of microwave irradiation and ionic liquid catalysts conducted in their work could also be tested in producing other reaction products. Another study (Khemthong et al., 2012) also tested the formation of biodiesel from palm oil using a microwave as a reactor. The catalytic testing demonstrated an enhanced biodiesel production compared to conventional heating. The maximum yield of fatty acid methyl esters reached 96.7% under the optimal condition of reaction time of 4 min with 900 W microwave power. Their results highlighted on the potential use of CaO catalyst for biodiesel production in a microwave.

A number of dehydrogenation reactions have been tested under a microwave for various product formation. For example, Ag-Rh nanoparticles were evaluated for production of H_2 during cyclohexane dehydrogenation at 300 °C. Upon experiment completion, it was found that the addition of Rh to Ag significantly synergized the H_2 evolution rate due to its excellent C-H bond cleavage ability. The highest H_2 evolution rate of 400 mmol/g_{Met}/min was achieved from the study (Pande et al., 2018). While de Oliveira et al. (2018) investigated effect of chromium content, and method of hydrothermal preparation of Cr/ZrO_2 catalysts on their catalytic properties for CO_2 oxidative dehydrogenation of propane. Their findings showed that the presence of CO_2 in the reactants causes a strong decrease of activity, selectivity, and yield towards propene. The results suggested Cr/ZrO_2 catalysts are potential catalyst for dehydrogenation of propane in the absence of CO_2. Bolotov et al. (2018) used microwave heating for ethylene selectivity by ethane dehydrogenation. It was found that $BaCl_2$–TiO_2–SnO_2 showed selectivity towards ethylene increase. Overall, the recent studies presented show good selectivity of various products by different catalysts when microwave is used under different conditions. This implies that microwave has a potential to be used for future research in chemical reactions.

2.5.1 Microwave background

The first application of microwave irradiation in chemical synthesis was published in 1986 (Kuhnert, 2002). The basic theory underlying the interaction of microwaves with macroscopic matter was formulated by von Hippel (Kuhnert, 2002). Over the last decade, microwave-assisted chemistry has matured into a highly useful technique and provides an interesting alternative for heating chemical reactions (Mirzaei and Neri, 2016).The first application of microwave heating to catalytic reaction was published by Jeff Wan in 1992 (Pineiro et al., 2017), and the technology has evolved to other applications in many catalysed reactions. More recently, improvements in commercial microwave ovens for organic synthesis allowed the controlled introduction of gases inside the reaction tube with consequent preparation of value added products via well-known catalytic synthesis, under moderate conditions and in some cases with higher reaction selectivities (Mercadante and Leadbeater, 2012).

Microwaves are electromagnetic waves having a wavelength that varies from 1 mm to 1m. Frequency of microwaves lies between 0.3 GHz and 3 GHz. A domestic microwave operates at 2450 MHz (a wavelength of 12.24 cm). Industrial/commercial microwave systems typically operate at 900 MHz (a wavelength of 32.68 cm) (Yin, 2012). Microwaves differ from conventional heating, because the waves provide gentle agitation that stirs the material for uniform heat distribution. This allows the microwave to apply all the heat directly into the reaction medium.

2.5.2 Microwave versus conventional heating

Traditionally, organic synthesis is carried out by conductive heating with an external heat source (e.g., an oil-bath or heating mantle). This is a comparatively slow and inefficient method for transferring energy into the system since it depends on effective heat transfer to the reacting molecules and the thermal conductivity of the reaction medium and vessel (Kappe et al., 2009). In a conventional heating, the direction of heating is from outside to inside of the solution resulting in higher temperature of the sample surface than the core, while for microwaves, the direction of heating is from inside to outside of the solution resulting in higher temperature of the sample molecule than the surface (see Figure 3). In contrast, microwave irradiation produces efficient internal heating by direct coupling of microwave energy with the molecules (solvents, reagents, catalysts) that are present in the reaction mixture.

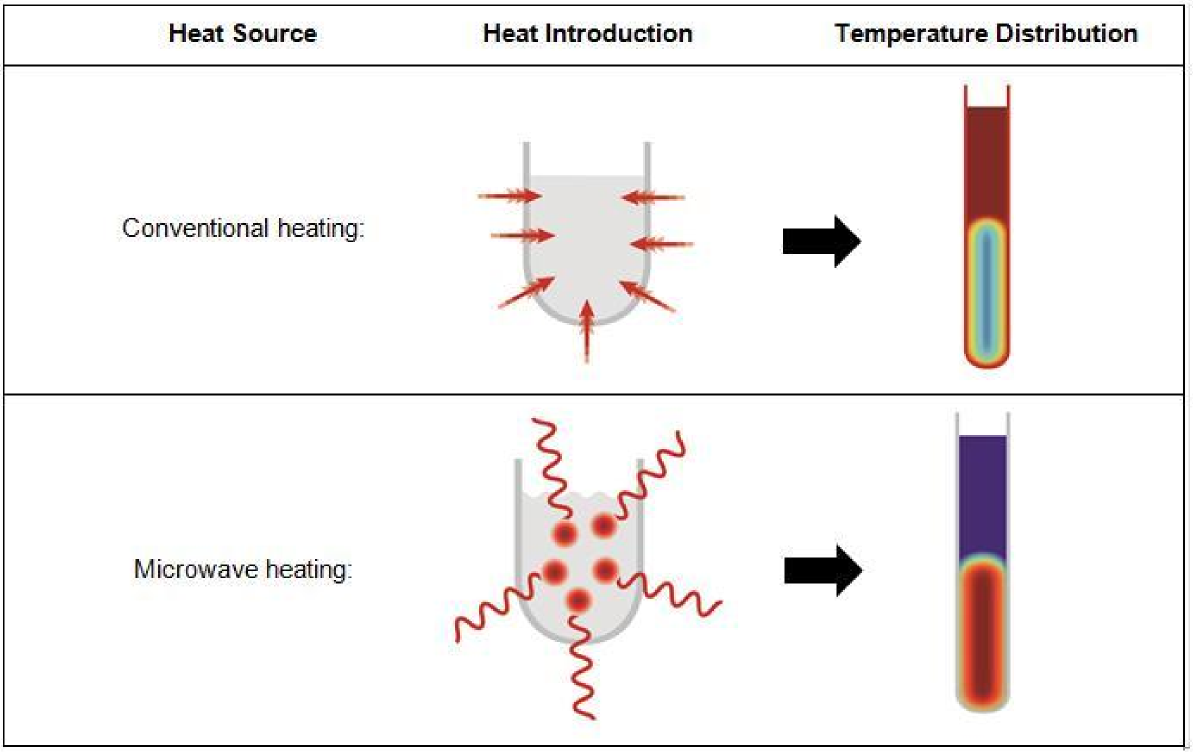

Figure 3 Microwave vs conventional heating (Kappe et al., 2009)

According to Kappe et al. (2009), activation energy is reduced when using a microwave in place of a conventional system. This is mainly due to rapid transfer of energy into the bulk of the reaction mixture occuring without inertia since only the product is heated. Furthermore, as the depth of penetration in materials is of the same order of magnitude as the wavelength, microwaves interact with substances of approximately 10cm thickness. By exposure to microwaves, the thermal effects undergone by materials exhibit an increased magnitude with the polarity increase of the substrate. These effects can appear in liquid systems, where structural modifications can also occur. In the presence of polar solvents, organic reactions require closed vessels to avoid volatilization. This leads to resultant advantages in comparison to conventional heating, since reactions are very rapid and usually complete after a few minutes, as a result of both temperature and pressure effects as well as the specific effects of the radiation (Deshayes et al.,1999).

2.6 Conclusion

Butanol has the potential to provide an alterantive energy that can offer many attractive features as transportation fuel. However, limitations of ABE have made processes such Guebert coupling very attractive in the butanol industry. Aldol condensation has been previously studied, but has not been studied extensively in terms of catalyst variation and reactor types. However, due to renewed interest of alternative fuels, many researchers are investigating more potential catalysts that can lead to higher butanol selectivity using conventional heating. Therefore, with all the literature key points stressed in the investigation, indicating the limitations and significance of considering different production routes of *n*-butanol as a fuel, it is vital to use this type of fuel in the near future and also investigate various methods for enhanced *n*-butanol yield.

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CHAPTER 3

Direct fermentation of sweet sorghum juice by *Clostridium acetobutylicum* and *Clostridium tetanomorphum* to produce bio-butanol and organic acids

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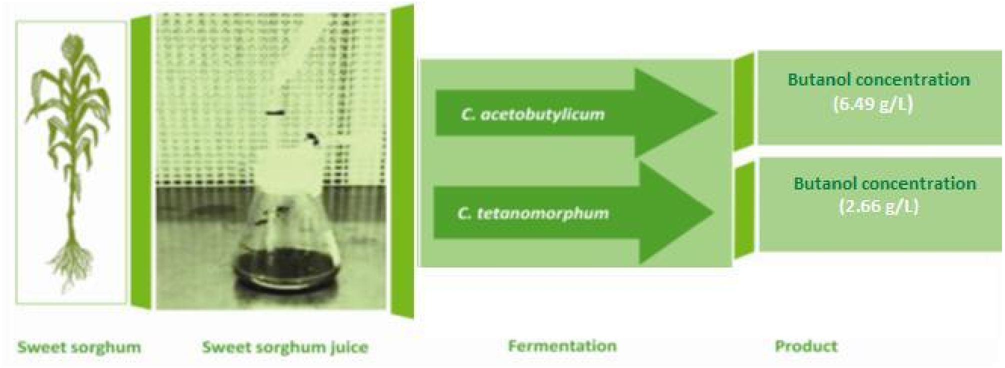
Graphical abstract



Abstract

Sweet sorghum juice was used as feedstock for a baseline study on the production of *n*-butanol from sugars through fermentation. Different inoculum concentrations of single Clostridial cultures (3, 5, 10 v/v %) were used to determine the effect of inoculum loading on *n*-butanol yield. Experiments were carried out in modified reactor flasks with a pH of 6-6.5, and incubated at 37˚C at 150 rpm shaking speed for 96 hrs. Fermentation products were analysed using high-performance liquid chromatography (HPLC). A maximum butanol concentration of 6.49 g/L (0.06 g/g of sweet sorghum juice) was obtained after 96 hrs of fermentation with 10 % v/v *C. acetobutylicum* with 98% reducing sugar conversion and butanol concentration of 2.66 g/L (yield of 0.02 g/g of sweet sorghum juice) was obtained at 5% v/v *C. tetanomorphum* with 97% reducing sugar conversion. Of all the acids and alcohols formed, *C. tetanomorphum* produced the highest concentration being butyric acid, while *C. acetobutylicum* mainly produced butanol. The results demonstrate a partially completed sugar conversion

with high butanol concentration in 96 hrs of fermentation when using sweet sorghum juice as a substrate. Additionally, these findings provide a basis for sugar rich syrup derived from sweet sorghum stem to be used as a renewable feedstock for butanol production.

3.1 Introduction

Bio-butanol has been considered as a better transportation fuel than bio-ethanol mainly due to its higher number of carbon atoms and consequently higher energy content, its miscibility in diesel, and higher blending capacity. There is a plethora of methods employed by the bio-chemical industries to produce bio-butanol. Over the past decades, direct fermentation of sugars derived from enzymatic conversion of starchy crops or by acid/enzymatic hydrolysis of lignocellulosic feedstock have dominated (Ranjan et al., 2013; Yang et al., 2014). In the butanol production processes, the production of other byproducts, e.g. acetone, ethanol, and acetic, butyric, and lactic acids, is undesirable; however, these byproducts are considered as main and valuable products in a number of processes (Zamani, 2015).

Sweet sorghum is one of the common feedstock for bio-butanol production. This is ascribed to the fact that it is grown in diverse climates; in both dry and wet areas (Goshadrou and Karimi, 2010). However, the application of this plant as biofuel feedstock has also been the subject of the famous food *vs.* fuel debate. To avoid such concerns, research has deviated from the starchy biomass to non-edible parts of the plant, i.e. the lignocellulosic biomass that could contribute to improving the socio-economic conditions of sweet sorghum as a dedicated energy crop. Fresh sweet sorghum comprises sugars, such as sucrose, glucose and fructose that can be extracted from the stalks.

According to Datta Mazumdar et al. (2012), Sobrinho et al. (2011) and Kundiyana et al. (2010), high quality sweet sorghum juice contains approximately 14-22 °Bx, but the values could vary depending on the soil origin of the plant. These sugars can be directly fermented using *Clostridium* spp. to produce ABE. This kind of fermentation involves two stages, namely; i) exponential growth stage (acidogenesis) and, ii) stationary stage (solventogenesis). During the acidogenesis, mainly acetic and butyric acids are produced while, during the latter, ABE solvents are generated (Borner et al., 2014).

Improvement on the cost effectiveness aspects of bio-butanol production has been widely investigated (Ni and Sun, 2009; Sillers et al., 2009). Several studies have strived to use different *Clostridium* spp. and biomass for the fermentation of sugars to bio-butanol (Ezeji et al., 2004; Kominkiat and Cheirsilp, 2013; Li et al., 2014). In addition to the cost issue, another way to minimize that expense is commercializing strains such as *Clostridium* spp. that utilize a diversity of substrates as well as making use of susbtrates derived from second generation biomass. The main aim of the study was to produce bio-butanol and organic acids using *C. acetobutylicum* and *C. tetanomorphum* from a low cost substrate (sweet sorghum juice) for enhanced butanol yield.

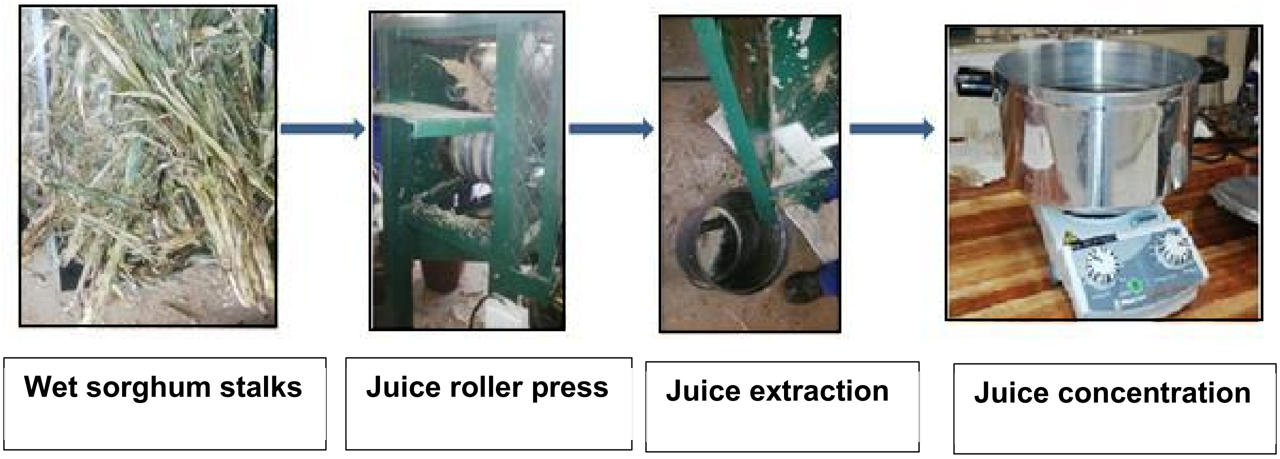
3.2 Materials and methods

3.2.1. Biomass

Sweet sorghum stalks were harvested in May 2013 at the test farm of the Agricultural Research Council – Grain Crops Institute of South Africa (ARC-GCI), Potchefstroom (26°43'43.16"S - 27°04'47.71"E). The juice was extracted from the stalks using a mechanical press roller. Approximately 4 L of the juice was extracted from 26.80 kg of fresh stalks and was stored at 4 °C until further use.

3.2.2 Long-term storage of the sweet sorghum juice

The prolonged storage protocol of sweet sorghum juice was adopted from Datta Mazumdar et al. (2012). Briefly, the juice was initially filtered using vacuum filtration to remove all the unwanted solid particles. Prior to heating, Brix and pH of the sample were determined using a refractometer and pH meter, respectively. The concentration of the juice was performed at 70 °C for 45 min on a hot plate with continuous stirring (see Figure 1). The heating was controlled to allow gradual temperature increases to avoid charring of the sugars. The juice was then cooled down to 40 °C resulting in clarified sweet sorghum juice of 73° Bx. Thereafter, the cooked juice was stored in Polyethylene terephthalate (PET) bottles at 4°C until further use.



Wet sorghum stalks

Juice roller press

Juice extraction

Juice concentration

Figure 1: Extraction and processing of sweet sorghum juice for long term storage

3.2.3 Strains and medium

C. *acetobutylicum* ATCC 824 and *C. tetanomorphum* ATCC 49273 were purchased from the American Type Culture Collection (ATCC). The stock cultures were maintained in the form of cell suspensions in 25% (v/v) sterile glycerol at -80°C. The organisms were grown on a Reinforced Clostridial Medium (RCM) for 24-48 hrs at 37°C before being sub-cultured into Clostridial Growth Medium (CGM), which was used to inoculate sweet sorghum juice for fermentation (Table 1). Both media were sterilised at 121°C for 15 min. Short-term stock cultures were prepared on *Clostridium* growth agar (CGA) and single colonies were revived every two weeks.

Table 1: The composition of the growth medium used.

Reinforced Clostridial medium (RCM)		**Clostridial growth medium (CGM)**	
Nutrients	Composition (g/L)	Nutrients	Composition (g/L)
Tryptose	10	NaCl	1
Beef extract	10	$(NH_4)SO_4$	2
Yeast extract	3	Yeast extract	5
Sodium chloride	5	KH_2PO_4	0.75
Sodium acetate	3	K_2HPO_4	0.75
Cystein hydrochloride	0.5	Asparagine	2
Soluble starch	1	$MgSO_4.7H_2O$	0.70
		$MnSO_4.H_2O$	0.01
		$FeSO_4.7H_2O$	0.01

3.2.4 Fermentation of sweet sorghum juice

Prior to fermentation, the concentrated sweet sorghum juice was diluted with distilled water to revert to the original Brix index of 14°Bx (16.3 g/L) (Sobrinho et al., 2011). High purity nitrogen (99.9 %) was sparged through the modified flasks before the inoculum addition to remove oxygen from the flasks and to maintain anaerobic conditions. The flask was modified using a tight rubber stopper to seal the opening, a micro-filter outlet, and sampling syringe to avoid closing and opening of the flask. A single colony from the plates was inoculated into 10 mL CGM and was incubated for 12 hrs. After incubation, the optical density at 600nm wavelength (OD_{600}) reached 0.798 and 0.810 for *C. tetanomorphum* and *C. acetobutylicum*, respectively. Fermentation was done by transferring specific volumes of the starter-culture (3, 5 or

10 % v/v) into the medium (sweet sorghum juice + nutrients) contained in the modified 250mL Erlenmeyer flasks with a 100 mL working volume.

During fermentation, temperature was maintained at 37°C, the pH was adjusted between 6 and 6.5 through the addition of NaOH or HCl, and the mixture was agitated at 150 rpm in a shaker incubator. Fermentation was conducted for 92 hrs and samples were taken at set time intervals during the experiment. All experiments were conducted in triplicates.

3.2.5 Analytical techniques used

Cell growth was analysed by measuring OD_{600} using a spectrophotometer (UV 7300, Jenway). The samples were then centrifuged using a ROTOFIX 32 centrifuge for 5 min at 5000 rpm and the supernatants were used to determine the concentrations of glucose, fructose and solvent after filtration with a 0.22-0.45 µm syringe filter. The reducing sugars, acids and solvent concentrations were measured using high performance liguid chromatography (HPLC) with an HPX-87H aminex column at 55°C refractive index detector (RID) and 30°C column temperature. The mobile phase used was 0.005 M H_2SO_4 at a flow rate of 0.6 mL/min with an injection volume of 5 µL.

3.3 Results and discussion

Sweet sorghum juice was subjected to fermentation with different cultures using *C. tetanomorphum* and *C. acetobutylicum*. The effect of different parameters on the fermentation of the juice containing 16.31 g/L sugars (6.25 g/L glucose, 0.31 g/L fructose and 9.75 g/L sucrose) was evaluated.

3.3.1 Fermentation with *C. tetanomorphum*

Fermentable sugars, i.e. glucose, fructose and sucrose, would normally undergo conversion to a range of intermediates after being consumed by the bacteria. It was observed that, after fermentation with *C. tetanomorphum*, the pH of the culture medium decreased from 6.5 to 4.6 as a result of organic acid formation. Single culture fermentation using different inoculum loadings (3, 5, 10 %v/v) of *C. tetanomorphum* was investigated for the production of bio-butanol. Figure 2 shows the effect of inoculum concentration on bio-butanol production within 96 hrs of fermentation. Even with 3 % inoculum, the optical density of the culture increased to more than 2 in the first 24 hrs of fermentation. Butanol production was accompanied by ethanol production, in the absence of acetone formation, which is consistent with the findings of Gottwald et al. (1984). Prior to the fermentation of sweet sorghum juice, broth medium-containing nutrients were inoculated with the organism and were incubated for 12 hrs. The initial optical density after 12 hrs of incubation and at 0 hr after the initiation of the juice fermentation was at 0.798.

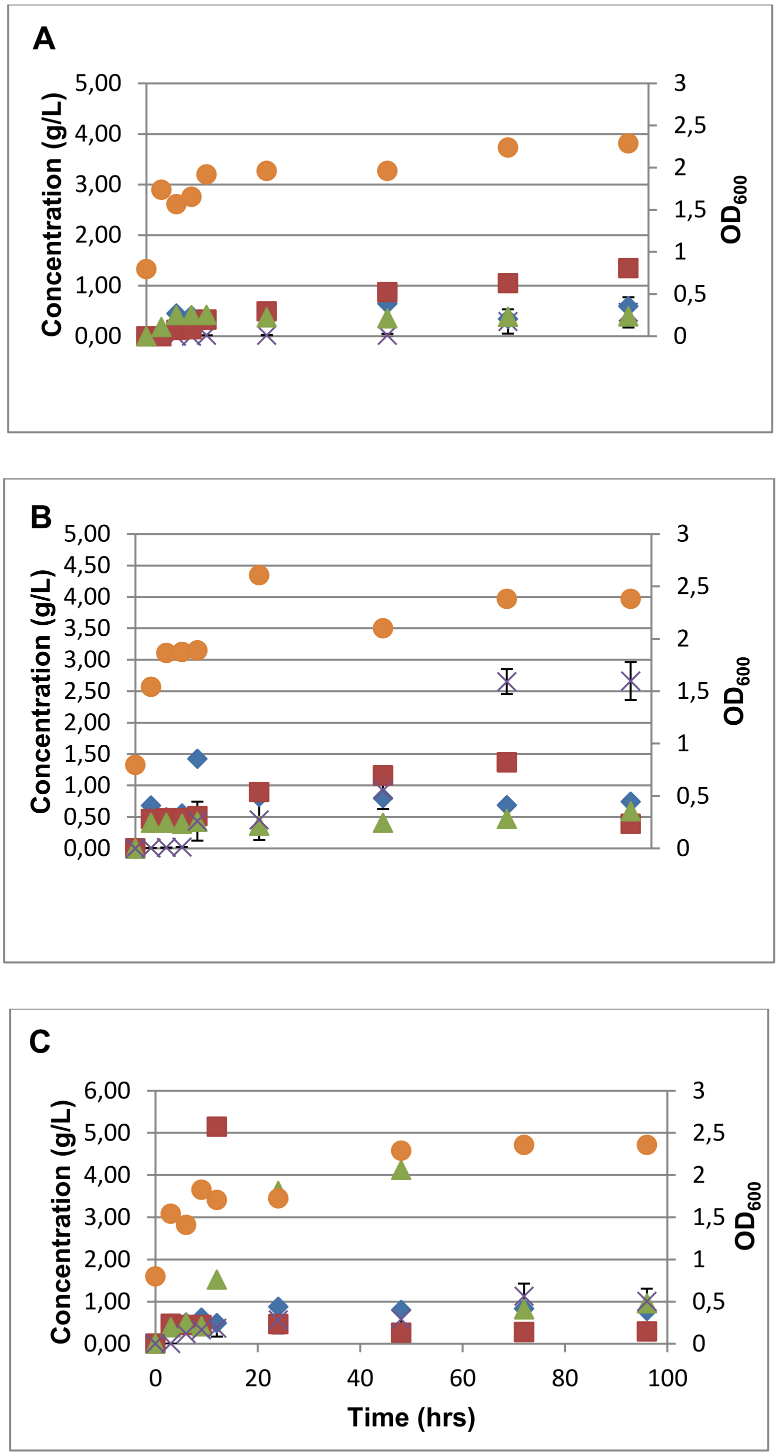


Figure 2: Effect of inoculum loading on acid and alcohol concentrations (■-Butyric acid, ♦-Acetic acid, ×-Butanol, ▲-Ethanol, ● - Cell density) for *C.tetanomorphum* inoculum concentrations of 3%v/v (A), 5%v/v (B), and 10%v/v (C)

Through the fermentation with *C. tetanomorphum*, organic acids, i.e. acetic and butyric acids, were initially generated followed by the production of alcohols, i.e. ethanol and butanol. The maximum acetic acid concentrations of 0.64, 1.43 and 0.87 g/L were obtained in the fermentation with 3, 5, and 10% *C. tetanomorphum* after 48, 12, and 24 hrs, respectively. In addition, the butyric acid concentration increased to 1.35, 1.37, and 5.15 g/L after 96, 72, and 12 hrs of fermentation with 3, 5, and 10% inoculum loadings, respectively.

With 3% inoculation, no detectable level of butanol was produced within the first 48 hrs of fermentation. The butanol concentration of less than 0.3 g/L and ethanol concentration of 0.42 g/L were obtained after 72 hrs of fermentation, which were produced mostly during the stationary phase. Organic acids showed a rapid increase after 12 hrs. Inoculating with 5 %v/v inoculum resulted in higher butanol and ethanol concentrations. During 96 hrs of fermentation, more than 2.5 g/L butanol and 0.5 g/L ethanol were produced. By increasing the initial inoculation volume to 10% of the medium, the final butanol and ethanol concentration increased to 1.14 and 0.96 g/L, respectively. However, the highest ethanol concentration was observed at 48 hrs of fermentantion with 10 %v/v *C. tetanomorphum*, subsequently followed by a sharp decrease. According to Eglinton et al. (2002), in the presence of acetic acid at high inoculum concentration, ethanol carbon sequestration takes place to regenarate NADH, leading to a vast decrease in ethanol concentration. No acetone was observed during fermentation with *C. tetanomorphum*. According to Fuyu et al. (2016); in nature, *C.tetanomorphum* produces ethanol and butanol without acetone. Butanol was the main alcohol produced during 5 % inoculation, whereas ethanol production prevailed when fermentation was conducted with 10 % inoculum. Optical density of the medium increased within the initial 12 hrs of the fermentation. Moreover, as a result of developing high concentrations of acids in the fermentation broth, the cell growth gradually decreased (Wu et al., 2013).

3.3.2 Fermentation with *C. acetobutylicum*

Sweet sorghum juice was subjected to fermentation with *C. acetobutylicum* at different inoculation ratios of 3, 5, and 10 % v/v for 96 hrs. The profiles of acids and alcohols as well as their respective concentrations and the optical density of the medium are shown in Figure 3. During the first 9 hrs of fermentation, sugars were utilised and fermented to acetic and butyric acids. After the organic acids concentration increased during the growth phase, alcohol formation was initiated by the cells during their stationary phase. The maximum acetic acid concentrations observed for 3, 5 and 10%v/v inoculation were 0.79, 0.63 and 0.89 g/L, respectively. Butyric acid with maximum concentrations of 0.63, 1.83, and 3.30 g/L was produced by using 3, 5 and 10%v/v inoculum, respectively.

The pH profiles of the media decreased from 6.5 to 4.1 through fermentation by *C. acetobutylicum.* These results were in line with those of Jiang et al. (2014), who reported rapid pH reduction during Clostridial fermentation indicating the formation of products. This may be attributed to the pH gradient of the cell membrane caused by increase of membrane fluidity. This causes a drastic modification in the pH, these modifications are pronounced and cause inhibition of sugar uptake especially in the presence of high concentrations of butanol.

A gradual increase in the concentrations of both alcohols was generally observed. By increasing the volume of the initial inoculum, the production of acids and alcohols was improved. Using 3 % inoculum of *C. acetobutylicum*, a maximum ethanol concentration of 0.56 g/L was obtained within 24 hrs. In addition, the maximum butanol and acetone concentrations of 1.09 and 0.12 g/L were obtained after 48 hrs. By increasing the inoculum loading to 5 and 10%, the ethanol concentration increased to more than 0.6 and 3.5 g/L, respectively. Moreover, by increasing the inoculum volume to 5%, the final butanol and acetone concentrations were increased to more than 1.9 and 0.3 g/L, respectively. A further increase in the inoculation volume resulted in higher butanol and acetone concentrations of 6.5 and 0.8 g/L, respectively.

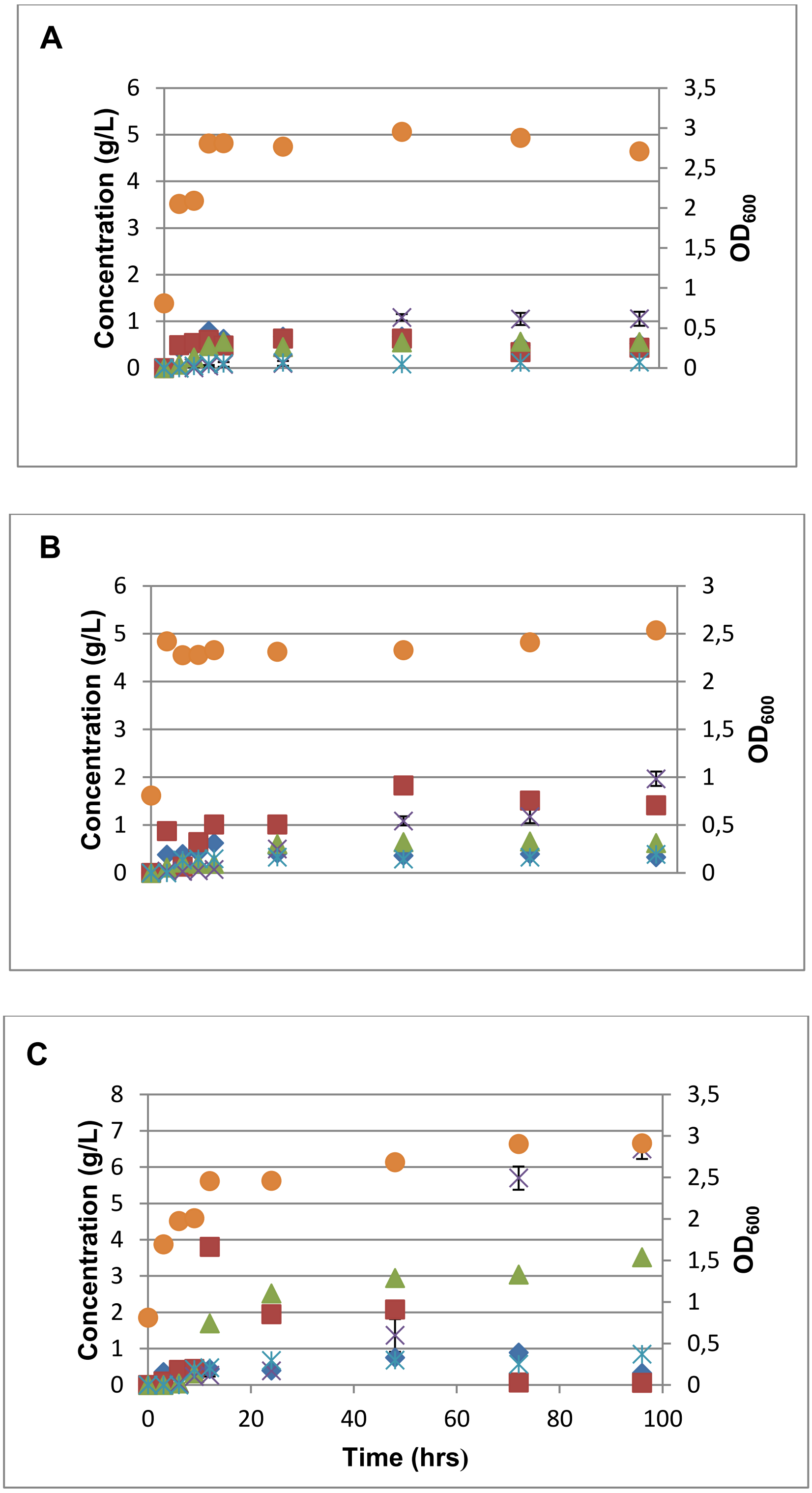


Figure 3: Effect of inoculum loading on acid and alcohol concentrations (■-Butyric acid, ♦-Acetic acid, ×-Butanol, ▲-Ethanol, ж-Acetone, and ●-Cell density) for *C. acetobutylicum* inoculum concentrations of 3%v/v (A), 5%v/v (B), and 10%v/v (C)

3.3.3 Total sugar conversion

Sugar consumption through the fermentation was highly dependent on the culture and its initial concentration. The profiles of sugar concentration during the fermentation with different cultures are presented in Figure 4. The sugars contained in the sweet sorghum juice (i.e. 6.25 g/L glucose, 0.31 g/L fructose, and 9.75 g/L sucrose) were consumed by the cultures, resulting in 0.3 to 2.5 g/L residual sugar. Through the fermentation with 10% v/v *C. acetobutylicum,* more than 98 % of the sugars contained in the juice were consumed.

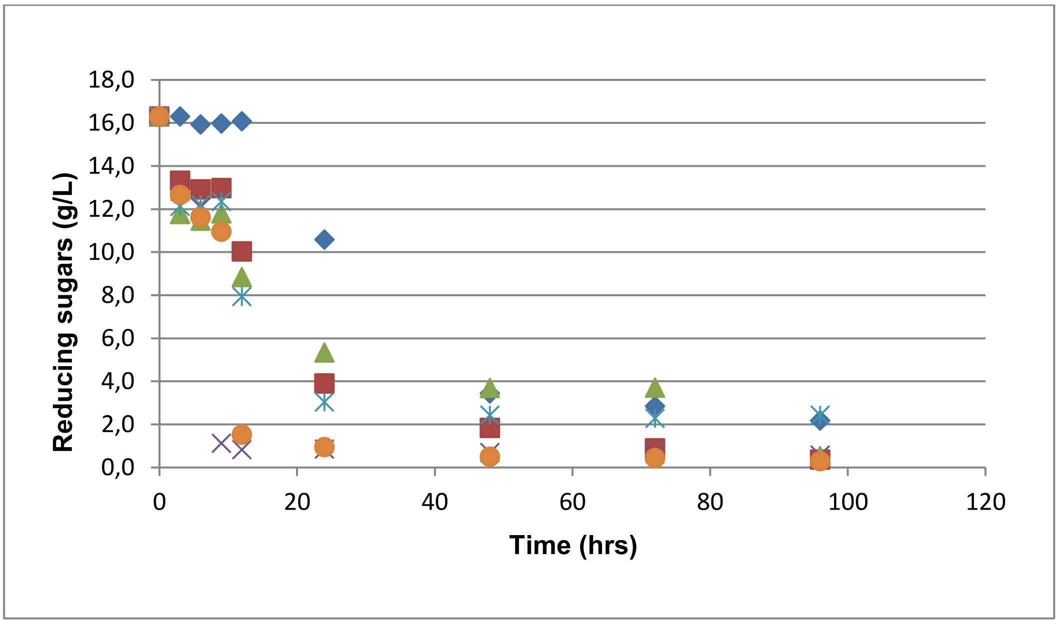


Figure 4: Total concentration of sugars (g/L) during the fermentation using different inoculum volumes 3 % (♦), 5% (■), and 10% (▲) *C. tetanomorphum* and 3% (×), 5 % (ж), and 10% (●) *C. acetobutylicum*.

In comparison with *C. tetanomorphum* fermentation, fermentation with *C. acetobutylicum* resulted in higher butanol production. Increasing the inoculum volume in the Clostridial fermentation improved the butanol production. The highest butanol concentration obtained was with 10% v/v *C. acetobutylicum* inoculation. On the other hand, through fermentation with 10% v/v *C. tetanomorphum,* the highest concentration of butyric acid was generated.

The best performances - in terms of total conversion of reducing sugars were obtained for 10% v/v *C. acetobutylicum* inoculation during fermentation (Figure 5).

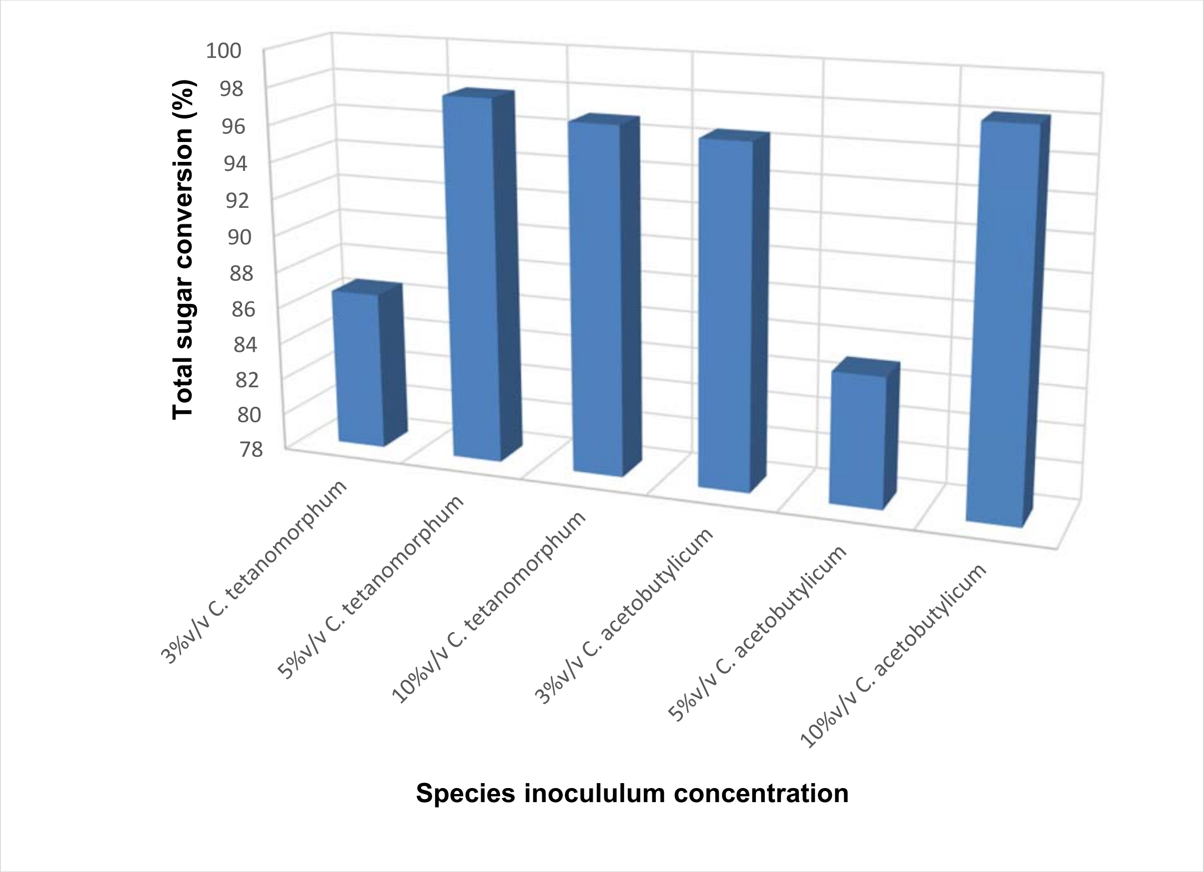


Figure 5: Total sugar conversion at different *Clostridium* species inoculum concentration

From Figure 5, it can be deduced that both species were able to convert sugars to a minimum of 80% conversion, with the maximum sugar conversion of 98% when 10%v/v *C. acetobutylicum* was used, followed by 97% when 5%v/v *C. tetanomorphum* was used. The highest sugar conversion at 10%v/v *C. acetobutylicum*, correlates with the highest butanol concentration of 6.49 g/L which was also formed during fermentation with 10%v/v *C. acetobutylicum*. However, the lowest sugar conversion of 85% was observed at 5%v/v *C. acetobutylicum* inoculum, and 86% of sugar conversion at 3%v/v *C. tetanomorphum* inoculum being the lowest when *C. tetanomorphum* was used. A major factor during the two fermentation which resulted in low sugar conversions was the acidogenesis stage which mainly produced butyric acid. During fermentation with 3%v/v *C. tetanomorphum*, it can be observed that acid concentrations were still increasing within the 96 hr, which translates to incomplete fermentation process, solventogenesis did not completely initiate. In contrast, during fermentation with 5%v/v *C. acetobutylicum*, acidogenesis stage was rapid, resulting in metabolic shift to solventogenesis. This lead to incomplete conversion of sugars to acids during acidogenesis stage. Incomplete conversion of sugars is generally influenced by a number of other factors such as sugar concentration, pH, hydrogen partial pressure, acetate, and butyrate are able to influence the growth rate, final products concentration and distribution of the products (Kong et al., 2006; Jo et al., 2008; Kumar et al., 2014). Overall, the reported results highlight that *C. acetobutylicum* was able to metabolize sugars typically present in sweet sorghum juice supplemented with the necessary nutritional factors.

3.4 Conclusion

Fermentation with *C. acetobutylicum* or *C. tetanomorphum* were evaluated for producing bio-based butanol from sweet sorghum juice. The results obtained showed that an inoculum loading of 10% v/v *C. acetobutylicum* resulted in the highest butanol concentration of 6.49 g/L (0.06 g/g of sweet sorghum juice). An inoculum loading of 10% v/v *C. tetanomorphum* was found to produce a high acid (butyric acid) concentration of 5.15 g/L (0.05 g/g of sweet sorghum juice), with 5% v/v producing the highest butanol concentration. These results have shown a great potential for use of *C. acetobutylicum* in producing bio-based butanol from sweet sorghum stalks' juice. However, there are a number of other parallel products which formed during fermentation. These include: acetone (0.85 g/L) and ethanol (3.52 g/L), leading to considerale low amounts of butanol. Additionally, low amounts of butanol could be due to incomplete conversion of acetic acid and butyric acid to form ABE. Another limitation could be that fermentation time (96 hrs) was not enough for further formation of bio-based butanol and complete susbtrate utilisation. To overcome such limitations, a chemical route for producing butanol was investigated in the subsequent chapter.

Additional notes

From the ABE fermentation results, further investigations using a different method is necessary for enhanced butanol yield. The experimental study in chapter 4 investigates different catalysts for production of butanol under a microwave reactor using bio-ethanol as a substrate. The bio-ethanol used in the study was purchased from Rochelle Chemicals, South Africa. Even though bio-ethanol can be produced from the laboratory, the current study required high volumes of bio-ethanol. The main challenge involved was obtaining anhydrous ethanol from a very dilute fermentation broth using a combination of distillation and adsorption. Microwave catalytic reactions in Chapter 4 were conducted using the bio-based ethanol from Rochelle Chemicals which was produced from sugarcane molasses. In conclusion, given continual research, development, and investment into butanol production technology, the study introduces a rapid microwave approach to obtain a high *n*-butanol yield from ethanol in shortened reaction times compared to ABE fermentation and conventional heating. This could lead to an accelerated entrance of *n*-butanol in the fuel market as an advanced drop-in fuel.

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CHAPTER 4

Rapid microwave-assisted conversion of bio-ethanol to *n*-butanol using metal oxide catalysts

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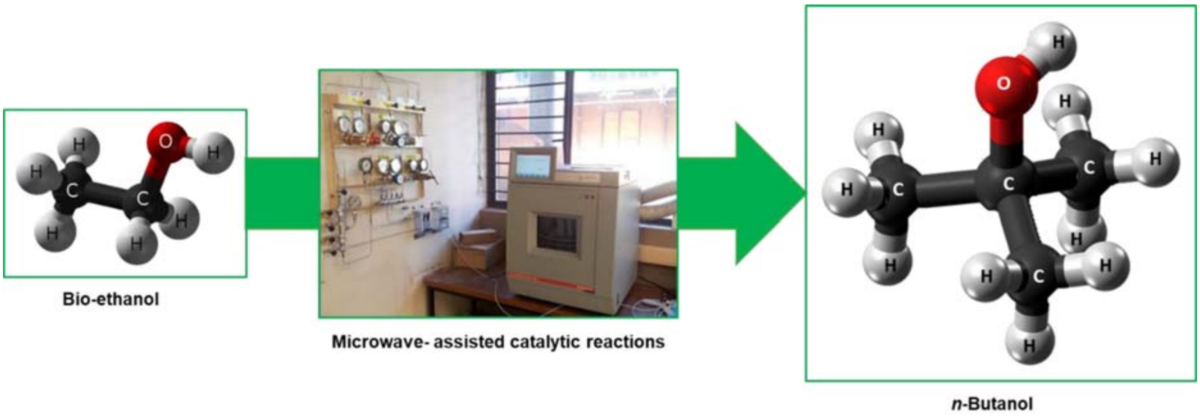
Graphical abstract



Abstract

Convenient approaches for butanol production have drawn significant attention. The study is aimed at investigating a microwave-assisted alternative method for conversion of bio-ethanol to *n*-butanol over metal oxide catalysts. Pre-screening of the catalysts used (MgO, Pt/Al_2O_3 and Ni/Al_2O_3) was conducted by a set of analytical techniques such as Brunauer-Emmet-Teller (BET) for determining the surface area, powder X-ray diffraction (P-XRD) for confirmation of structural pattern, thermogravimetric (TGA) analysis for observing thermal stability and transmission electron microscopy (TEM) for observing catalyst morphology. A 2-level factorial design with three variables, reaction temperature (200 °C and 250 °C), catalyst loading (1 and 5 wt.%) and reaction time (30 and 60 min) was applied to investigate the effect of the catalysts on ethanol conversion and butanol selectivity. Quantitative analysis by high performance liquid chromatography (HPLC) showed that Ni/Al_2O_3 performed the best in terms of *n*-butanol selectivity (58%) and bio-ethanol conversion (20.8wt.%) compared to the

Pt/Al_2O_3 catalyst which achieved an *n*-butanol selectivity of 11.5% at a 9.2 wt.% bio-ethanol conversion in 30 min. The MgO catalyst produced mostly acetaldehyde with a concentration of 140 g/L (0.13 g/g of bio-ethanol) as a main reaction product at a bio-ethanol conversion of 10.7 wt.%. Thus, Ni/Al_2O_3 catalyst was selected for further investigations. The effect of reaction time on *n*-butanol yield was investigated at 250 °C with a Ni/Al_2O_3 catalyst loading of 5 wt.%. The highest *n*-butanol concentration achieved was 161 g/L (0.22 g/g of bio-ethanol) with 60% selectivity towards butanol at a 76.6 wt.% conversion of ethanol in 60 min reaction time. This study showed, for the first time, that the use of microwave technology can not only shorten the reaction time (from 8 h to 1 hr), but also achieve both high selectivity towards butanol and high ethanol conversion at relatively low reaction temperatures. These results present a method for the production of a drop-in fuel from biomass that is both economically and technically feasible and environmentally responsible.

Keywords: *n*-Butanol; microwave; catalyst; bio-ethanol; yield.

4.1 Introduction

The global biofuels industry is growing; however, there are a few challenges that still need to be resolved. One of the solutions for these challenges is the development of safe and convenient methods for the production of biofuels such as bio-based butanol. An increase in bio-based butanol yield is of particular importance in order to make bio-based butanol production an economically feasible approach. The economic feasibility of butanol production is depending on raw material and production cost. Thus, the development of butanol production process using bio-based sources is pivotal (Ghaziaskar and Xu, 2013).

Synthetic chemists have neglected alternative sources of heat for chemical reactions, and still rely primarily on conventional reactors for producing *n*-butanol (Ghaziaskar and Xu, 2013; Dziugan et al., 2015; Pereira et al., 2015; Lim et al., 2016). On the other hand, biologists have focused on the use of different bacteria, and how those bacteria can be modified to enhance bio-based butanol concentrations (García et al., 2011; Isomaki et al., 2017; Ibrahim et al., 2018). However, the challenge of reaching maximum butanol yield using ABE fermentation or conventional heating seems to still persist.

Several species of *Clostridium* bacteria are capable of metabolizing different sugars, amino and organic acids, and other organic compounds to butanol and other solvents. Butanol being of relatively high value is usually the most desired product. Depending on the type of strains and substrates used in the fermentation, the optimal fermentation conditions (pH, temperature, and nutrients), fermentation products and the ratio of their formation vary. A number of Clostridial species have been investigated (Ellis et al, 2012, Abd-Alla and El-Enany, 2012; Gottumukkala et al., 2013). These species target high conversion of glucose to butanol, but a limited by product inhibition with a butanol tolerance of 13 g/L (García et al., 2011). This means that these strains are unable to produce butanol concentrations beyond the 13 g/L threshold. To make butanol production a viable technology it is not advisable to rely on the current ABE fermentation, unless method development is employed to enhance butanol concentration.

The catalytic conversion of ethanol to butanol process is an alternative to the direct fermentation of sugars to butanol. Since ABE fermentation has limitations, including low butanol yield and by-product (acetone and ethanol) formation, upgrading ethanol to butanol in order to increase the value of short-chain alcohols has been intensively studied (Gholizadeh, 2010; Riittonen et al., 2012; Uyttebroek et al., 2015). Butanol synthesis proceeds via dehydrogenation of ethanol in the presence of a metal catalyst to form acetaldehyde which then couples with itself to further form other reaction intermediates such as butyraldehyde and ethyl acetate which eventually form *n*-butanol (Ramasamy et al., 2016).

The catalytic reaction is usually carried out in the gas phase and is catalyzed by basic or bifunctional acidic–basic metal oxides (Ndou et al., 2003). Strontium and calcium hydroxyl phosphates (hydroxyapatites) are reported to be promising catalytic materials for the reaction. The addition of transition metals like Pd/Cu/Ni increases the conversion rates by accelerating the dehydrogenation step (Ho et al., 2016). However, there is still a need for innovation and improvement in terms of selectivity and activity of the catalysts.

Another novel approach is to produce *n*-butanol from ethanol in a liquid phase where ethanol molecules interact with the catalyst surface before undergoing aldol condensation and side reactions. For the liquid phase reactions using alcohols such as methanol, ethanol and 2-butanol, the turnover rates are normally lower than those in the gas phase reaction (Liu et al., 2018). This can be attributed to increased pressure which causes detrimental effect of water on conversion and selectivity (Gabriëls et al., 2015). Although this is generally caused by a deactivating effect on the catalyst, water could also influence the thermodynamic equilibrium of the aldol condensation. It might slow down the desired reaction pathway, thereby leaving intermediate aldehydes unreacted and available for other, undesired parallel reactions (Sreekumar et al., 2014; Gabriëls et al., 2015).

However, depending on the catalysts, remarkable effects on the improvement of product selectivity can be achieved. Catalysts applied include zeolites-based catalysts and supported metal oxides (e.g. Ru, Ni, Co) for the catalytic conversion of ethanol to *n*-butanol (Wingad et al., 2015). Amongst these, Ni supported on alumina catalysts has shown excellent catalytic activity in hydrogenation reactions (Jimenez-Gonzalez et al., 2013; Ayodele et al., 2014; Riittonen et al., 2015). In recent investigations, nanoscale catalysts have also been shown to positively affect the conversion of ethanol to butanol. Nanoscale catalysts possess features such as high surface area and stability which may improve reaction conversion and selectivity towards *n*-butanol (KrishnaKumar and Imae, 2014).

The ethanol to butanol reaction makes use of conventional heating in a stainless steel autoclave operated at temperatures ranging from 250 °C to 450 °C and high pressures with long reaction times (3-7hrs) with conversion ranging from approximately 10–20% and 80% selectivity (Jordison et al, 2016; Riittonen et al., 2012). Intense research interest is pursued to develop technologies with reduced reaction times without compromising catalytic reaction conversion and butanol yield (Dowson et al., 2013; Ho et al., 2016).

The increased use of microwave technology (Kure et al., 2017) in organic synthesis lead to microwave-assisted catalytic investigation in the current study. The benefits of the microwave technology have drawn increasing interest, because of convenient heating method for chemical reactions in comparison to conventional heating systems, thereby reducing reaction times. Microwave heating has been proven to improve the reaction selectivity in terms of the main product yield and limiting the formation of by-products (Wiesbrock et al., 2004; Mirzaei and Neri, 2016).

Irradiation of the sample at microwave frequencies results in the dipoles or ions aligning in the applied electric field. As the applied field oscillates, the dipole or ion field realign itself with the alternating electric field and, during that process, energy is lost in the form of heat through molecular friction and dielectric loss (Kappe, 2004). This process is mainly dependent on the ability of a specific material (solvent or reagent) to absorb microwave energy and convert it into heat. The rapid increase in temperature can be more prominent for media that has extreme loss factors, such as ionic liquids, where temperature can escalate to 200 °C within a few seconds. In essence, such temperature profiles within short contact times are very difficult if not impossible to achieve when standard thermal heating is employed.

In this study, a one-step microwave technology was used to convert ethanol to *n*-butanol at reduced reaction times. To the best of our knowledge, there is no existing report on bio-ethanol conversion using MgO, Pt/Al_2O_3, and Ni/Al_2O_3 catalysts on a batch microwave. These catalysts were evaluated for their ability to produce *n*-butanol under different temperatures and catalyst loading. Consequently, Ni/Al_2O_3 catalyst was selected to further investigate the effect of microwave time (5 to 60 min) at fixed catalyst loading and temperature of 250 °C on catalyst selectivity, product distribution and *n*-butanol yield using bio-ethanol as feed.

4.2 Materials and methods

4.2.1 Materials

Bio-ethanol (purity 99%) was purchased from Rochelle Chemicals, South Africa. Catalyst containing 20%Ni on Al_2O_3 was purchased from Riogen, United States of America (USA). MgO (99.9%) and 5% Pt on Al_2O_3 were purchased from SIGMA-ALDRICH. The catalysts were stored in air tight containers and placed in a desiccator until use. Nitrogen gas (N_2) (99.9%) was purchased from Afrox, South Africa and used for purging and pressurizing the reaction vessels. Acetaldehyde (99.5%), butyraldehyde (99.5%), and acetic acid (98%) were purchased from Merck, South Africa and used to determine the presence and concentration of by-products formed.

4.2.2 Microwave-assisted reaction experiments

An Anton Paar Multiwave PRO 3000 industrial microwave with 8 XQ80 high-quality reaction vessel quartz (40 mL working volume) was used for all experiments. Operational maximum temperature and pressure of the microwave are 300 °C and 80 bar. All three catalysts were activated in a furnace at 700 °C for 1 hr prior to flushing with nitrogen gas for microwave experiments. A two level factorial design with three variables (2^3) was used to determine the significant parameters that influence the reaction and evaluate the performance of different catalysts (see Table 1). This was done using Design Expert software version 8.0.7.1. In a typical experiment, the required catalyst (MgO, Pt/Al_2O_3 or Ni/Al_2O_3) was loaded (1 or 5 wt% based on mass of ethanol used) into the reaction vessel and mixed with 99% bio-ethanol. The choice of catalysts used in this study were selected based on their activity from previous studies conducted (Ndou et al., 2013; Ghaziaskar and Xu, 2013; Sabour et al., 2016).

Table 1: 2^3 factorial design variables

Factor A	Factor B	Factor C
Temperature (˚C)	**Catalyst loading (wt%)**	**Time (min)**
250	5	60
250	1	60
250	5	30
200	1	60
200	5	30
200	1	30
250	1	30
200	5	60

XQ80 high-quality quartz sample holder vessels were loaded with samples, sealed tightly, placed inside the microwave system, purged and loaded with N_2 gas while inside the microwave for safety precautions. A temperature probe was used to measure the temperature during experiments. Microwave temperature was set and average pressure of the vessels was detected during the reaction. Ramping (temperature to 250 ˚C) and cooling (from 250 ˚C-55 ˚C) time for microwave temperature were 45 and 30 min, respectively. After the completion of the process, the XQ80 vessels were taken out of the microwave system and were allowed to cool down at room temperature. The reaction products were quantitatively transferred from the reaction vessels into centrifuge vials and the vessels were rinsed with 10 mL 99% acetone. The liquid product was then filtered with 0.2 µm filters and diluted with 0.005M H_2SO_4 (1000x dilution) for HPLC analysis.

During the evaluation of the effect of time on the product yield, the reaction temperature was kept constant at 250 °C and reaction time was varied (5-60 min with

5 min increments). The starting nitrogen pressure was below 10 bar for each reaction, but increased thermodynamically as the reaction temperature increased with 80.8 bar being the maximum pressure reached. All reaction mixtures were cooled from 250 to 55 °C before product analyses were performed. The catalyst which produced optimum butanol yield was further investigated for the effect of it's re-usability in producing *n*-butanol. The catalyst was examined at 250 °C with a feed of bio-ethanol. Prior to re-use cycle of the experiment, the catalyst was separated by centrifugation, washed with deionised water, and then oven dried over night at 110 °C. This reaction was conducted for three successive reactions which were conducted in 30 min steps.

4.2.3 Analytical techniques

4.2.3.1 High Performance Liquid Chromatography (HPLC)

The reaction products were quantitatively analysed using HPLC analysis (BioRad; Aminex HPX-87H column, with column temperature of 30 °C, Refractive Index (RI) detector temperature of 55 °C, injection volume: 10 µL; Flow rate 0.6.mL/min, and 0.005 M H_2SO_4 as mobile phase. A standard set of calibration curves were used to quantify each component in the reaction mixture.

4.2.3.2 Powder X-ray diffraction (P-XRD)

Powder X-ray diffraction (P-XRD) (Bruker D2 Phaser, Stellenbosch University) was operated at 10 mA current and 30 kV voltage with a monochromatic CuKα radiation (λ= 1.5405Å) with a 2θ step of 0.05/sec, with a scan range of 2θ = 20° - 80°. The obtained results were further processed in Origin 9 software. The characterization was done in order to observe the crystalline regions and also check the purity of each catalyst.

4.2.3.3 Transmission electron microscopy (TEM)

The catalyst morphology and size were examined using TEM. Prior to analysis, the sample was sonicated in 99% ethanol for 15 min and deposited on a copper grid. Transmission Electron Microscopy Phillips Tecnai 12 model operated at 200 kV with a point resolution of 0.23 nm.

4.2.3.4 N_2 adsorption analysis

The pore volume and surface area of the catalysts were measured by BET analysis (Micromeritics ASAP 2020). The samples were degassed with nitrogen gas for 2 hrs at 250 °C before analysis. Nitrogen adsorption/desorption isotherm values were measured at -196.15 °C. The pore size distribution was calculated from desorption branch of the isotherm by the Barrett, Joyner and Halenda (BJH) method (Shiraz et al., 2016).

4.2.3.5 Thermogravimetric analysis (TGA)

TGA was used in order to monitor the rate of change in the weight of all three catalysts as a function of temperature. The weight change of each catalyst was monitored by thermogravimetric analysis (TGA) using an instrument model TGA-Q500 V6.7 Build 203 in nitrogen flow of 40 mL/min). The samples were heated from room temperature up to 600 °C at a heating rate of 10 °C/min (Chiang et al., 2015).

4.3 Results and discussion

4.3.1 Catalyst screening

Prior to conducting experiments, all three catalysts (MgO, Pt/Al_2O_3, and Ni/Al_2O_3) were characterised by a set of analytical techniques in order to better understand their structural characteristics and relate to their behaviour. The surface area (S_{BET}) and pore volume (V_P) of the catalysts were determined using BET analysis (see Table 2).

Table 2: Bet analysis results

Catalyst type	Surface area (S_{BET}) m^2/g	Pore volume (V_P) cm^3/g
MgO	57.71	015
Pt/Al_2O_3	123.12	0.21
Ni/Al_2O_3	158.57	0.39

The surface area and pore volumes are similar to those reported by Riittonen et al., 2012; Camtakan et al., 2012; Suresh et al., 2014. Transmission electron microscopy (TEM) confirmed the spherical shape of the Ni/Al_2O_3 catalyst particles as shown in Figure 1 (A). The nickel particles (black spots) are dispersed on the Al_2O_3 support. The size distribution of the catalyst particles is in the range of 3-8 nm. The catalyst particles are in the form of slightly spherical shape. This kind of observation is akin to those reported in studies conducted by Goula et al., 2015 and Fu et al., 2016. TEM micrographs of MgO are shown in Figure 1(B). The application of TEM showed that the morphology of the particles was hexagonal-shaped (dark spots). Figure 1 (C) shows evenly dispersed cubic platinum crystals on alumina.

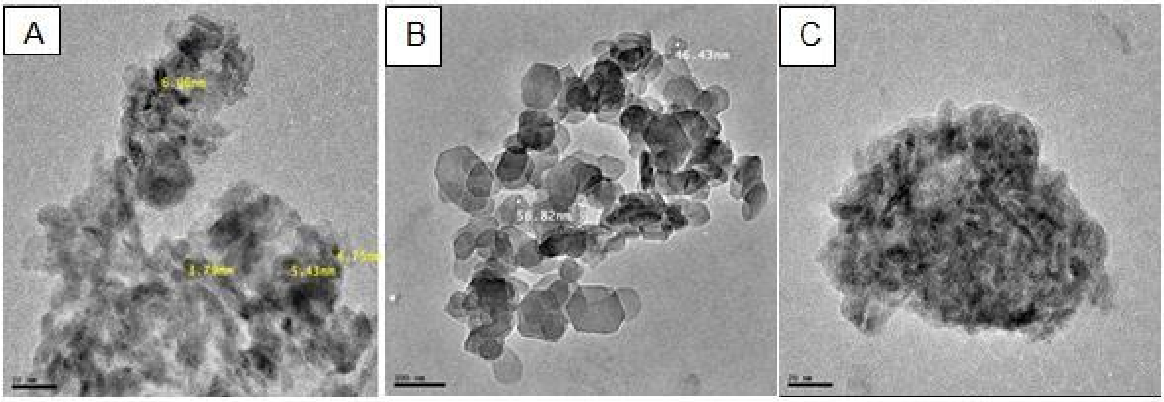
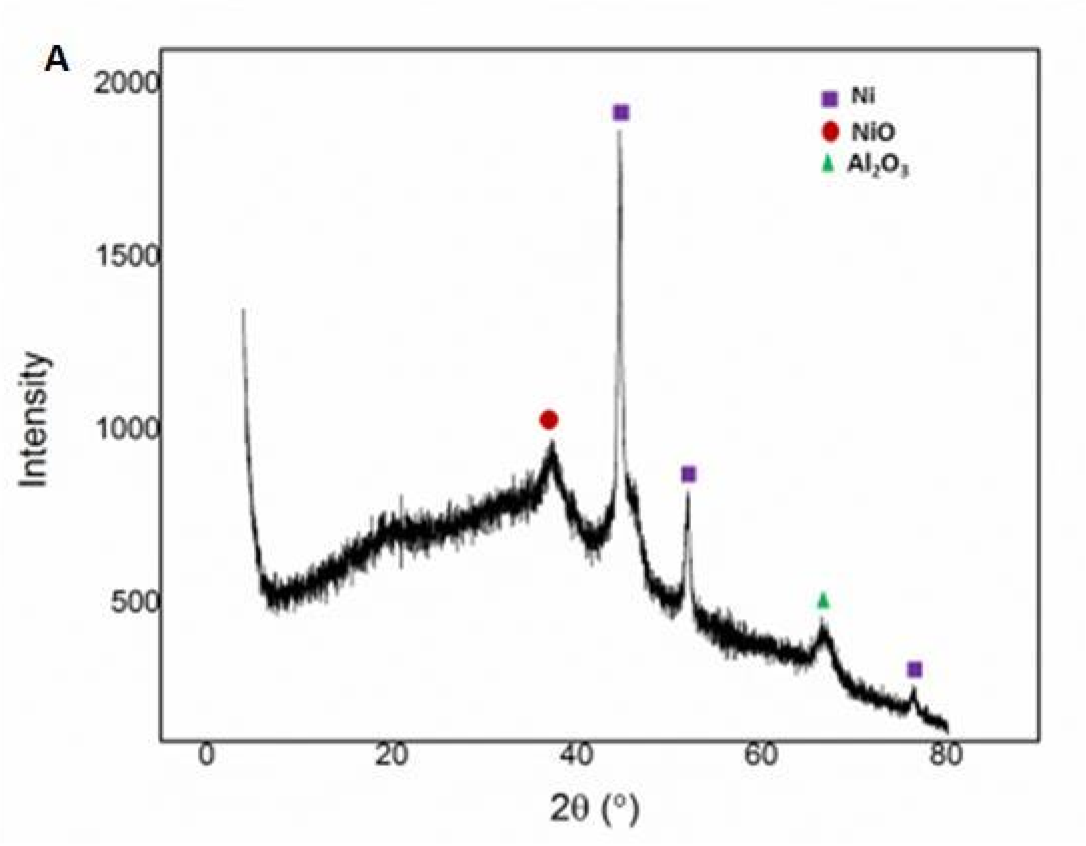


Figure 1: TEM images for (A) Ni/Al_2O_3, (B) MgO and (C) Pt/Al_2O_3 catalysts

Powder X-ray diffraction determines the crystalline structure of the nickel on alumina catalyst as presented in Figure 2 (A). The X-ray diffraction pattern shows five characteristic peaks of the Ni on alumina crystalline structure at 2θ = 38.1°, 43.3°, 52.1°, 66.9° and 78.2°. The appearance of NiO peak at 38.1° could be related to the remnants of NiO during reduction process (incomplete thermal treatment) to elemental Ni. The Ni diffraction peaks are found at 43.3°, 52.1° and 78.2°. The intensity of the peak is consistent with a 20% presence of Ni in the catalyst, and this is also in agreement with results obtained by Zangouei et al. (2010). The Al_2O_3 characteristic peak is present at 66.9° which confirms the catalyst support composition. Figure 2 (B) represents the XRD pattern for MgO. The observed sharp peaks at 2θ=50 and 74° are attributed to the presence of the crystalline region of a face-centred cubic structure of MgO. The peak at 43° might be due to small amounts of residual Mg $(OH)_2$ during

MgO preparation (Suresh et al., 2014). The XRD pattern of Pt/Al_2O_3 is presented in Figure 2 (C). Sharp peaks of Pt catalyst were detected at 40, 47, and 68°, these peaks can be attributed to a Pt metallic phase. The peak at 33° is associated with octahedral position of β-Al_2O_3 present in the sample (Gobora et al., 2014). Overall, the results confirm the purity and contents of the studied catalysts.



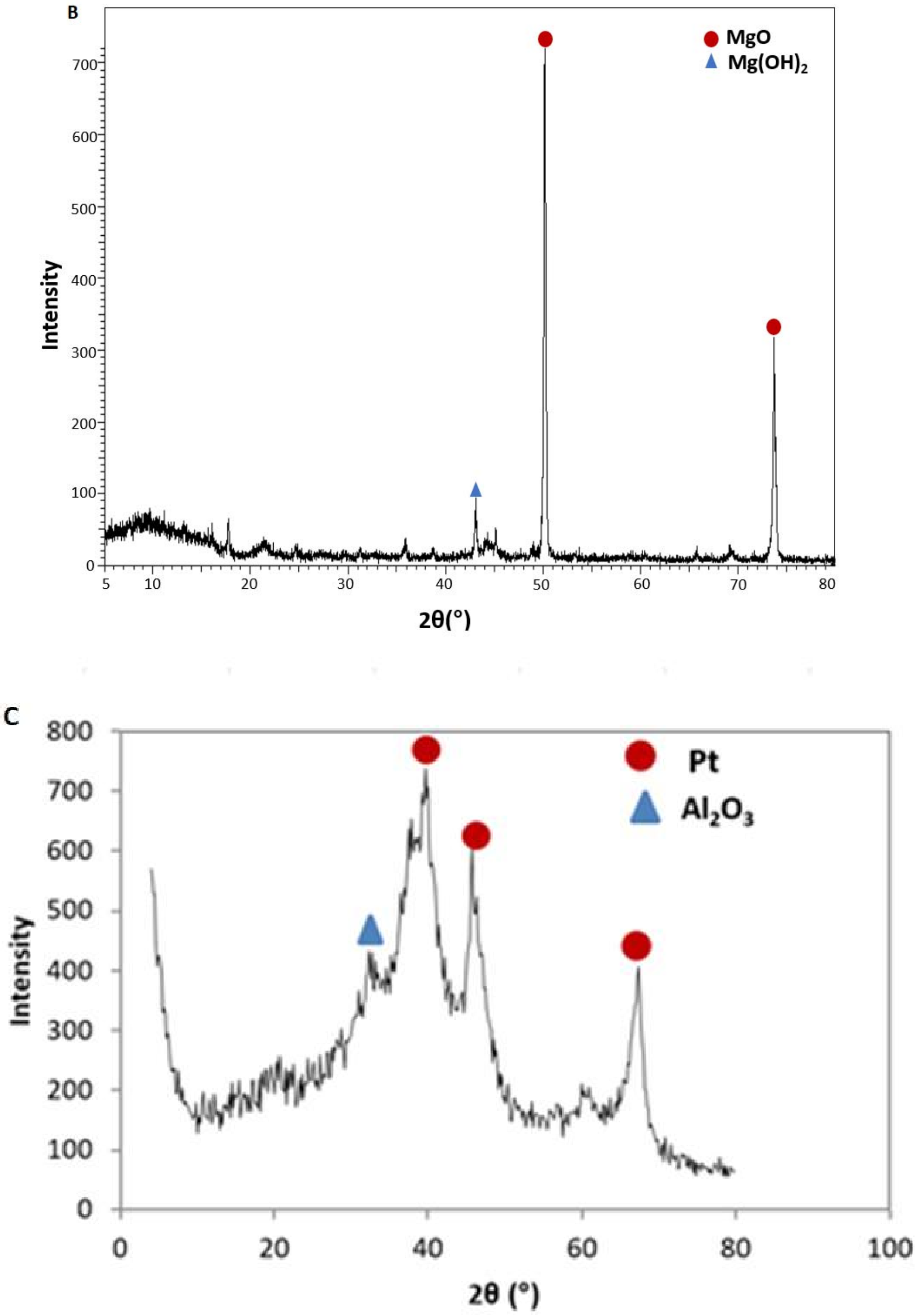
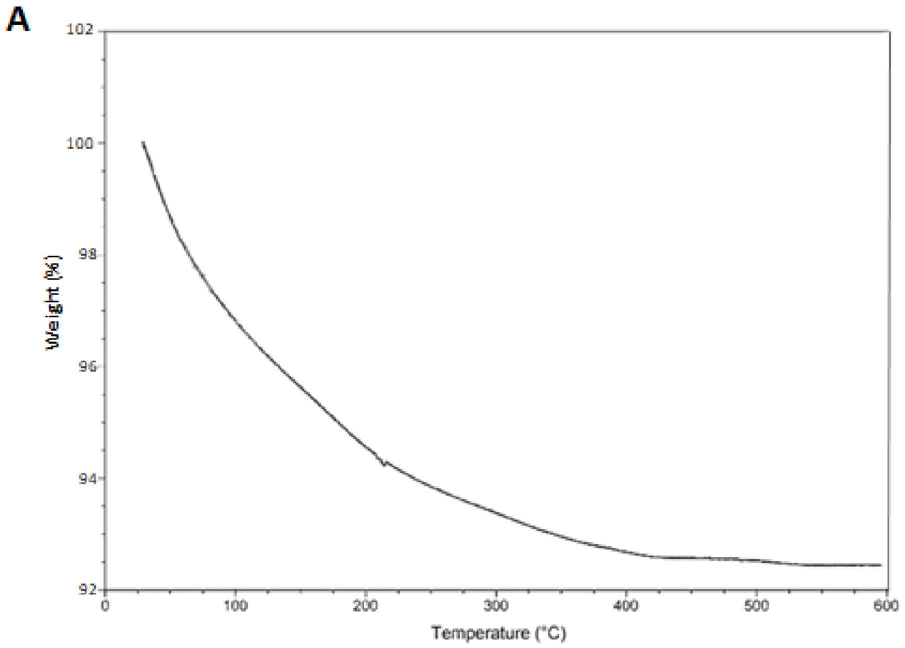
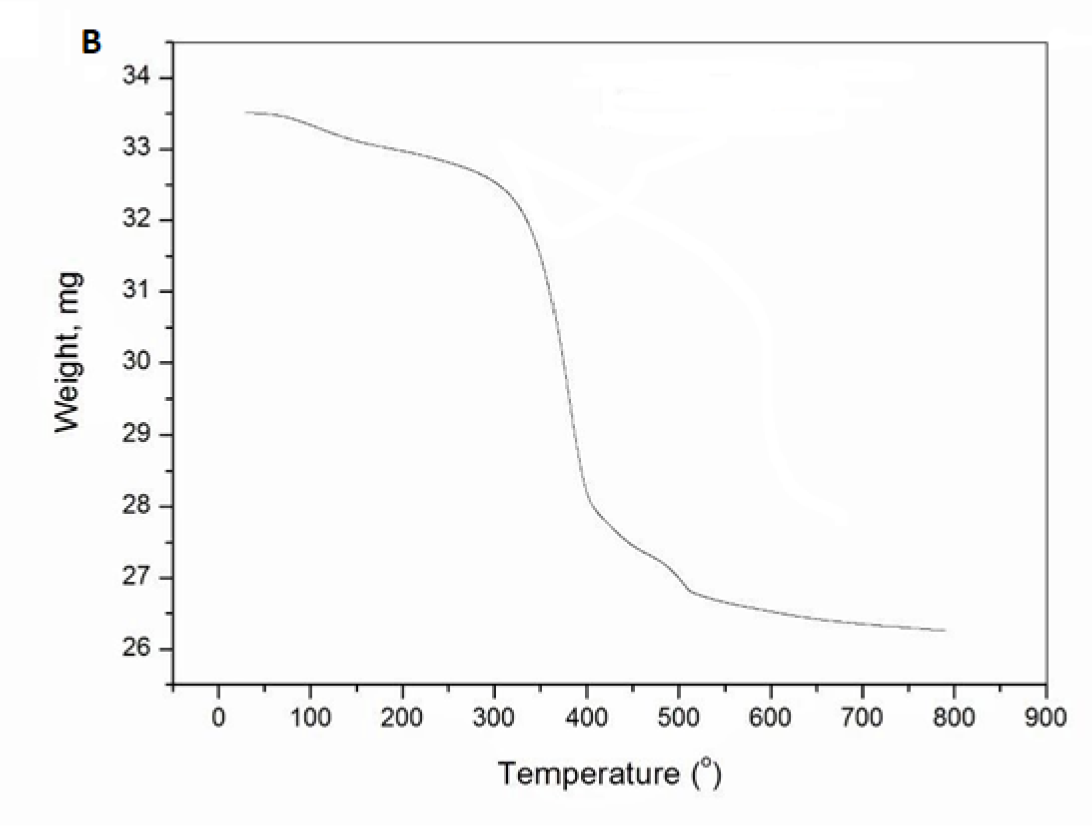


Figure 2: XRD patterns for (A) Ni/Al_2O_3, (B) MgO, and (C) Pt/Al_2O_3 catalysts

The thermal behaviour of the three studied catalysts as obtained by TGA is presented in Figure 3. The thermographs presented in Figure 3 (A, B, C) show a gradual mass loss of 7.5, 20.9, and 26% for Ni/Al_2O_3, MgO, and Pt/Al_2O_3, respectively. This could be assigned to the organic solvent, bulk and physisorbed water. In DTA profile (see Figure 3A), an endothermic reaction at temperature region 100 ºC was attained. This is attributed to water molecules removal from the alumina support. The sharp peak after 200 ºC shows the decomposition of nickel nitrates which are embedded in the surface pores of the catalyst (Zangouei et al., 2010). Figure 3 (B) illustrates two main phases of catalyst weight loss. The first weight decrease is observed around 300 ˚C corresponding to removal of surface water. The second decrease from 400-500 ˚C, is attributed to the elimination of volatile materials in the catalyst (Santhosh et al., 2015). Pt/Al_2O_3 was also analysed with TGA to reveal the changes that occurred during heat treatment. According to the curve presented in Figure 3 (C), the major part of weight loss appeared below 100-200˚C, this is also attributed to the removal of absorbed water in the catalyst. Another loss occurred between 300- 500˚C, this is linked to decomposition of residual materials (Gobora et al., 2014)





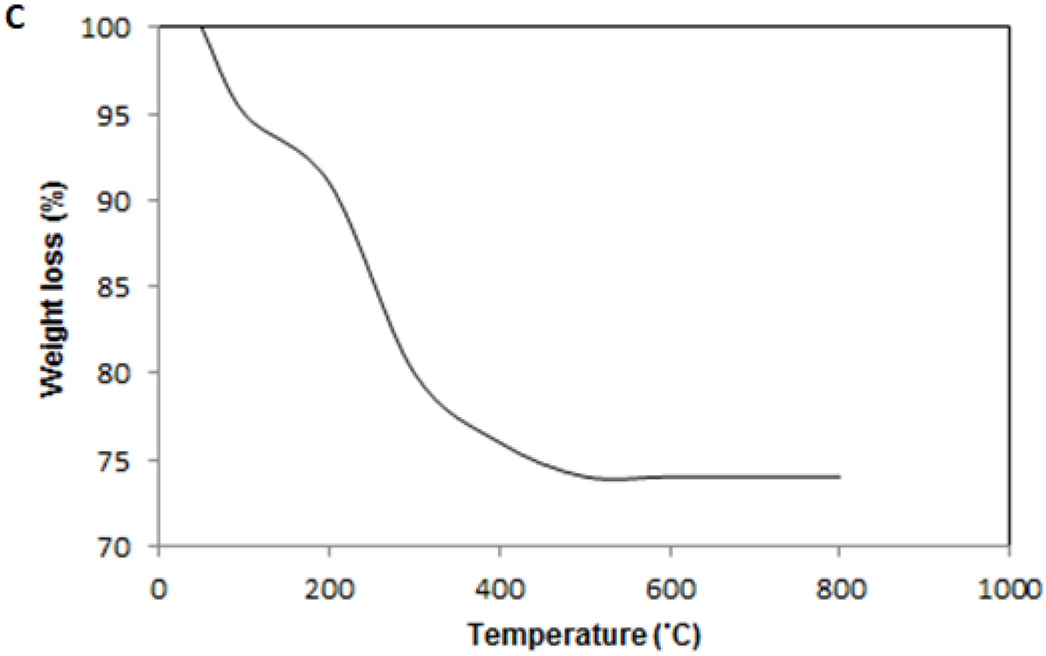


Figure 3: TGA profiles of (A) Ni/Al_2O_3, (B) MgO, and (C) Pt/Al_2O_3 catalysts

Among the three characterized catalysts, Ni/Al_2O_3 had the largest surface area, and purity. Generally, a higher surface area and purity is associated with high catalyst activity (Cox, 1999). The performance of all three catalysts was tested for the conversion of ethanol to *n*-butanol using a 2-level factorial design.

4.3.2 Parameter screening by 2-level factorial design

A single step microwave catalytic conversion process was conducted for the conversion of bio-ethanol to *n*-butanol. The 2-level factorial design of experiments was used to screen the performance of the catalysts and determine the most significant variables that influence the conversion. Variable reaction parameters were two (200 °C and 250 °C, reaction time (30 and 60 min) and catalyst loading (1 and 5 wt%). The performance of all three catalysts varied, this could be attributed to their different structural characteristics. Effect of varying the reaction parameters in bio-ethanol conversion and *n*-butanol yield is presented in Table 3.

Table 3: Factorial design (2^3) for the significant factors affecting butanol yield and bio-ethanol conversion when using different catalysts

Factor A	**Factor B**	**Factor C**	**MgO**		**Pt/Al$_2$O$_3$**		**Ni/Al$_2$O$_3$**	
Temperature (˚C)	*Catalyst loading (wt%)*	*Time (min)*	*n-butanol yield (g/g)*	*Bio-ethanol conversion (wt%)*	*n-butanol yield (g/g)*	*Bio-ethanol conversion (wt%)*	*n-butanol yield (g/g)*	*Bio-ethanol conversion (wt%)*
250	5	60	0.001	10.68	0.011	9.19	0.121	41.8
250	1	60	0.004	10.18	0.002	8.25	0.055	15.15
250	5	30	0.001	14.2	0.006	9.19	0.105	20.7
200	1	60	0.002	14.9	0.001	5.7	0.001	5.3
200	5	30	0.003	5.7	0.003	11.9	0.004	5.5
200	1	30	0.002	14.5	0.003	12.3	0.005	11.3
250	1	30	0.003	10.4	0.00	8.1	0.017	10.6
200	5	60	0.008	14.2	0.017	9.4	0.035	20.8

From table 3 it can be deduced that different catalysts produce different *n*-butanol yields at different controlled parameters. The highest butanol conversion and yield were found when Ni/Al$_2$O$_3$ was used at a temperature of 250°C. The results presented in Figure 4 depicts the effect of catalyst type on by-product concentrations.

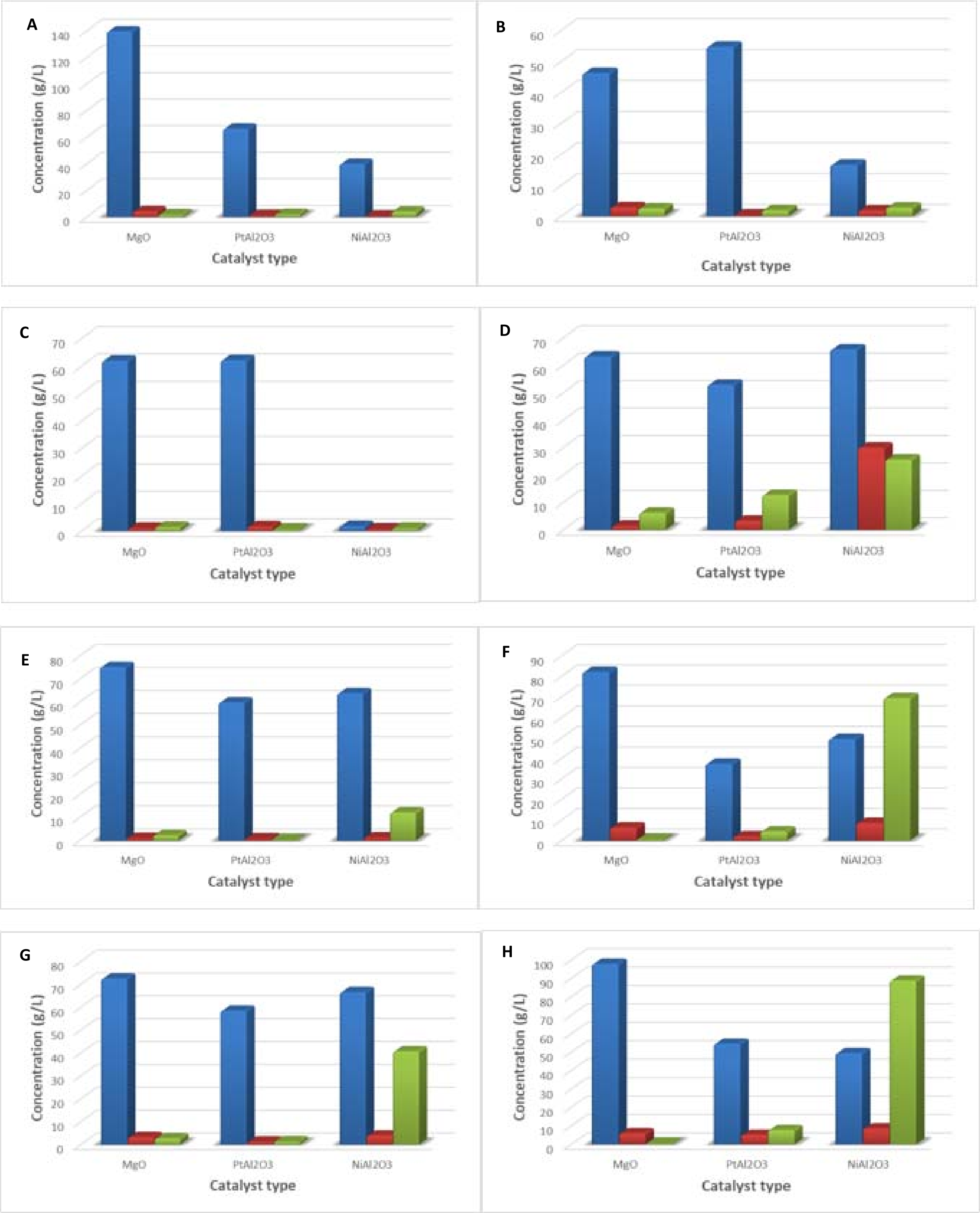
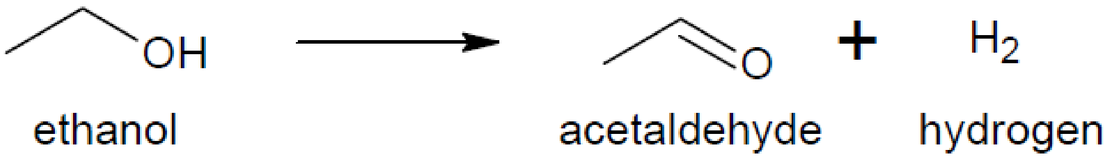


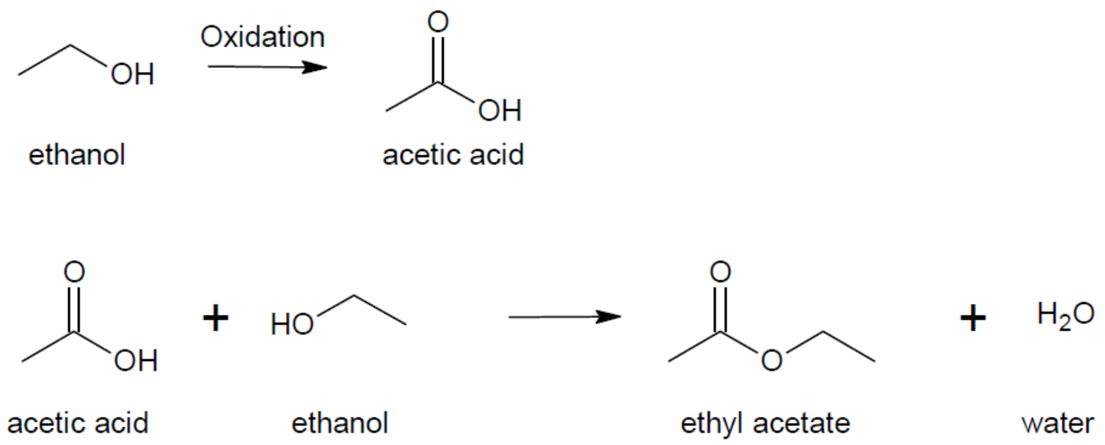
Figure 4: Effect of catalyst type on product concentration (🟦- acetaldehyde,🟥- butyraldehyde, 🟩- *n*-butanol) at 200°C: catalyst loading of 1wt% (A), 5wt% (B) in 30 min and catalyst loading of 1wt% (C), 5wt% (D) in 60 min and at 250°C: catalyst loading of 1wt% (E), 5wt% (F) in 30 min and catalyst loading of 1wt% (G), 5wt% (H) in 60 min.

Formation of by-products during organic reactions is not new, as this has been previously studied (McMurry and Simanek, 2007; Riitonen et al., 2012). The most prominent product formed during the microwave-assisted catalytic conversion of ethanol to *n*-butanol was acetaldehyde (140 g/L) in all three catalysts tested, but mainly produced when MgO catalysts was tested at 200°C. However, MgO produced the lowest yield of *n*-butanol. This suggests that MgO was catalysing the dehydration of ethanol but inefficient at catalysing the aldol step during the reaction. Therefore, any acetaldehyde formed could not react further to form *n*-butanol. The Pt/Al_2O_3 catalyst also produced low yields of *n*-butanol, but it did contribute to high yields of acetaldehyde, with the highest found to be 66 g/L. This was not the case for Ni/Al_2O_3 as acetaldehyde formed and consequently produced high butanol concentration (89 g/L) compared to MgO and Pt/Al_2O_3.

Acetaldehyde is produced through dehydrogenation of ethanol (Scheme 1), it could also be a result of hydrogen formed during the reaction. However, hydrogen was not measured in this study as the commercial batch microwave reactor limited gas analysis during the reaction. Acetic acid formation observed when all three catalysts were used, even though it formed in negligible amounts (not shown). Formation of acetic acid is possible during side reactions that could form water as a by-product. In addition, it is possible that acetic acid was observed due to incomplete reaction of ethanol to form ethyl acetate (which was not detected in the HPLC), because acetic acid being carboxylic acid further produces an ester (ethyl acetate) via oxidation of the alcohol (Scheme 2). It should also be noted that these catalysts are also good oxidation catalysts (Ndou et al., 2002, Siankevich et al., 2018; Yue et al., 2018), and this could explain the formation of acetic acid. Riittonen et al. (2012) proposed a side reaction on the possible formation of acetic acid to form ethyl acetate, and this is shown in the Scheme 2.



Scheme 1 Dehydrogenation of ethanol to form acetaldehyde



Scheme 2 Two-step reaction forming acetic acid which further converts to ethyl acetate

The highest butyraldehyde formed when Ni/Al_2O_3 was used at 250°C. Butyraldehyde also forms during dehydrogenation of ethanol. According to Jordison et al. (2016), butyraldehyde forms in high amount at temperature below 350°C, but decreases at temperatures above 350 °C. The ANOVA results for *n*-butanol yield for the two-level factorial design are given in Table 4, 5, and 6. ANOVA results were determined at a 95% confidence level and therefore all factors with a $p<0.05$ were considered to have a significant effect.

Table 4: Response for MgO catalyst

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	0,0001	7	0,0000	388,00	< 0.0001	***significant***
A-Temperature	0,0000	1	0,0000	256,00	< 0.0001	
B-Catalyst loading	2,667E-06	1	2,667E-06	64,00	< 0.0001	
C-Time	0,0000	1	0,0000	400,00	< 0.0001	
AB	0,0001	1	0,0001	1444,00	< 0.0001	
AC	8,167E-06	1	8,167E-06	196,00	< 0.0001	
BC	4,167E-06	1	4,167E-06	100,00	< 0.0001	
ABC	0,0000	1	0,0000	256,00	< 0.0001	
Pure Error	6,667E-07	16	4,167E-08			
Cor Total	0,0001	23				

Std. Dev.= 2.65, R-Squared=0.9235, Mean= 21.43

Table 5: Response for Pt/Al_2O_3 catalyst

Source	***Sum of Squares***	***df***	***Mean Square***	***F-value***	***p-value***	
Model	*0,0006*	*7*	*0,0001*	*122,79*	*< 0.0001*	***significant***
A-Temperature	*9,375E-06*	*1*	*9,375E-06*	*12,50*	*0,0027*	
B-Catalyst loading	*0,0003*	*1*	*0,0003*	*420,50*	*< 0.0001*	
C-Time	*0,0001*	*1*	*0,0001*	*180,50*	*< 0.0001*	
AB	*0,0001*	*1*	*0,0001*	*180,50*	*< 0.0001*	
AC	*3,375E-06*	*1*	*3,375E-06*	*4,50*	*0,0499*	
BC	*3,750E-07*	*1*	*3,750E-07*	*0,5000*	*0,4897*	
ABC	*0,0000*	*1*	*0,0000*	*60,50*	*< 0.0001*	
Pure Error	*0,0000*	*16*	*7,500E-07*			
Cor Total	*0,0007*	*23*				

Std.Dev.=2.622E-003,R-Squared=0.8844,Mean=5.375E-003

Table 6: Response for Ni/Al_2O_3 catalyst

Source	*Sum of Squares*	*df*	*Mean Square*	*F-value*	*p-value*	
Model	*0,0371*	*7*	*0,0053*	*12,72*	*< 0.0001*	***significant***
A-Temperature	*0,0181*	*1*	*0,0181*	*43,43*	*< 0.0001*	
B-Catalyst loading	*0,0089*	*1*	*0,0089*	*21,25*	*0,0003*	
C-Time	*0,0049*	*1*	*0,0049*	*11,76*	*0,0034*	
AB	*0,0020*	*1*	*0,0020*	*6,89*	*0,0137*	
AC	*0,0033*	*1*	*0,0033*	*7,28*	*0,0291*	
BC	*0,0018*	*1*	*0,0018*	*6,83*	*0,0136*	
ABC	*0,0029*	*1*	*0,0029*	*6,92*	*0,0182*	
Pure Error	*0,0067*	*16*	*0,0004*			
Cor Total	0,0438	23				

Std. Dev.= 0.013, R-Squared= 0.9666, Mean= 0.043

From Table 4, it can be observed that all factors and factor interactions investigated had a significant effect on the *n*-butanol concentration. Significant in this context means that all factors affect butanol concentration, but that does not imply a high concentration since an effect can be positive, negative, smaller or large. Despite all the values being significant, MgO produced the lowest butanol yield in all the tested conditions compared to the other catalysts tested in this study. A correlation between the low yields and low surface area of the MgO particles could be established. According to Mnguni et al. (2012), the decrease in yield for the catalysts calcined at 700 and 800˚C could be explained by the formation of other substances which could possibly hinder the reaction and lead to low butanol yields, due to unwanted remainants in the catalyst. This concurs with the current results for MgO which was calcined at 700˚C for activation. Ndou et al. (2003), also stated that it is possible for MgO to not improve butanol yield, the low yields can also be attributed to the activation temperature used. The low yields obtained suggests that reaction parameters could be significant and yet low butanol yields could still be formed.

Table 5 represents the response model for results obtained using the Pt/Al_2O_3 catalyst. The model F-value of 12.79 implies the model is significant. The interaction effect of catalyst loading and time were not significant (p value-0.4897). This implies that the performance of Pt/Al_2O_3 is not influenced by the amount (catalyst loading) of the catalyst at a certain reaction time at a given temperature. Considering the mechanism of formation of Pt/Al_2O_3 intermetallides, Petraszek et al. (2007), showed that, during the first step of the reaction, Pt^{4+} ions could be bound to surface hydroxyl groups. Then, they can interact with aluminium ions at the octahedral positions of β-Al_2O_3 as shown by XRD results of the current study. The reduction of Pt^{4+} ions followed by the reduction and clustering of aluminium ions finally results in the formation of cationic vacancies on the surface of aluminium oxide leading to the formation of the butanol.

Table 6 shows the response model for results obtained when using the Ni/Al_2O_3 catalyst. The model was reported as significant with all the factors and interactions significant. The highest amount of butanol was obtained when temperature and catalyst loading were kept constant with an increase in time. The results proved the significance of interaction of all the 3 factors (temperature, catalyst loading, and time) on butanol yield in the presence of Ni/Al_2O_3 catalyst. Even though, it is scientifically proven that temperature plays an important of any catalyst activity. Temperature is not the only phenomena that controls the reaction temperature, temperature along with other factors, in this case catalyst loading and time, governs the overall heat of reaction, which ultimately decides the product yield (Patel and Patel, 2018). Different studies reveal that conversion and yields depend on the type of metal, metal loading, preparation method, nature of support and the presence of additives in the catalyst. Ni was found to exhibit better catalytic activity in terms of ethanol conversion and butanol yields compared to Pt over γ-Al_2O_3 support. The role of active metal is to activate ethanol and promote its reaction with the hydroxyl group generated due to dissociation of water molecule on oxide support. These catalyst surface phenomena were corroborated by analysis of the catalyst by XRD, TEM, and BET, which revealed high purity and dispersion of active metal by providing surface area. According Dhanala et al. (2015), support also plays a significant role in the reaction. Comparing Ni/Al_2O_3 and MgO, both catalysts were found to have significant parameter interaction. However, MgO produced the lowest yield of butanol compared to ethanol. The optimum values were validated by selecting the optimal values predicted by the model. In this case, continuous investigations for conditions of the Ni/Al_2O_3 catalyst by studying the effect of residence time on *n*-butanol, acetaldehyde, butyraldehyde, acetic acid and bio-ethanol concentration were conducted.

4.3.3 Catalyst choice

4.3.3.1. Effects of residence time using Ni/Al_2O_3

Based on the presented results in Table 2, Ni/Al_2O_3 was chosen for further bio-ethanol to *n*-butanol conversion. The effect of residence time (from 5-60 min with 5 min increment) in the presence of Ni/Al_2O_3 catalyst for bio-ethanol to *n*-butanol conversion was studied. The experiments were performed at a fixed temperature of 250 °C. The presented results in Figure 7 show four product yield distribution from the reaction. These include butanol, acetaldehyde, butyraldehyde and acetic acid. Figure 8 represents bio-ethanol conversion and butanol yield at different reaction times.

4.3.3.1.1. Product distribution

The reactions were performed in a microwave system at a fixed temperature of 250 °C with time ranging from 5 to 60 min using ethanol obtained from sugarcane molasses. A fresh Ni/Al_2O_3 catalyst was used in the reaction. A total of four major compounds were traced and identified by HPLC after every reaction (*n*-butanol, acetaldehyde, butyraldehyde, and acetic acid). It was noticed that the butanol concentration increased with an increase in reaction time. At 5 to 45 min, an extremely low butanol concentration was obtained, whereas at 60 min, high concentration of butanol of 161 g/L (0.22 g/g of ethanol) was observed. An increasing trend of acetaldehyde conversion occurred up to approximately 113 g/L (0.16 g/g of ethanol), with an increase in reaction time before 35 min and followed by a sharp decrease to 52 g/L (0.07g/g) after 35 min. This occurred as a result of the dehydrogenation of ethanol to the first form of acetaldehyde before *n*-butanol formation during the reaction. The formation of butyraldehyde and acetic acid maintained a constant conversion, which was less than 5%. Acetic acid, however, increased slightly to 44 g/L (0.06 g/g of ethanol) at 60 min. The microwave reactor method converts bio-ethanol into other forms of products faster than the conventional method, which requires long reaction time in hrs. Nonetheless, the microwave method has a limitation of 60 min reaction at 250 °C, due to a maximum pressure limit for the microwave; therefore, the reactions were discontinued after 60 min.

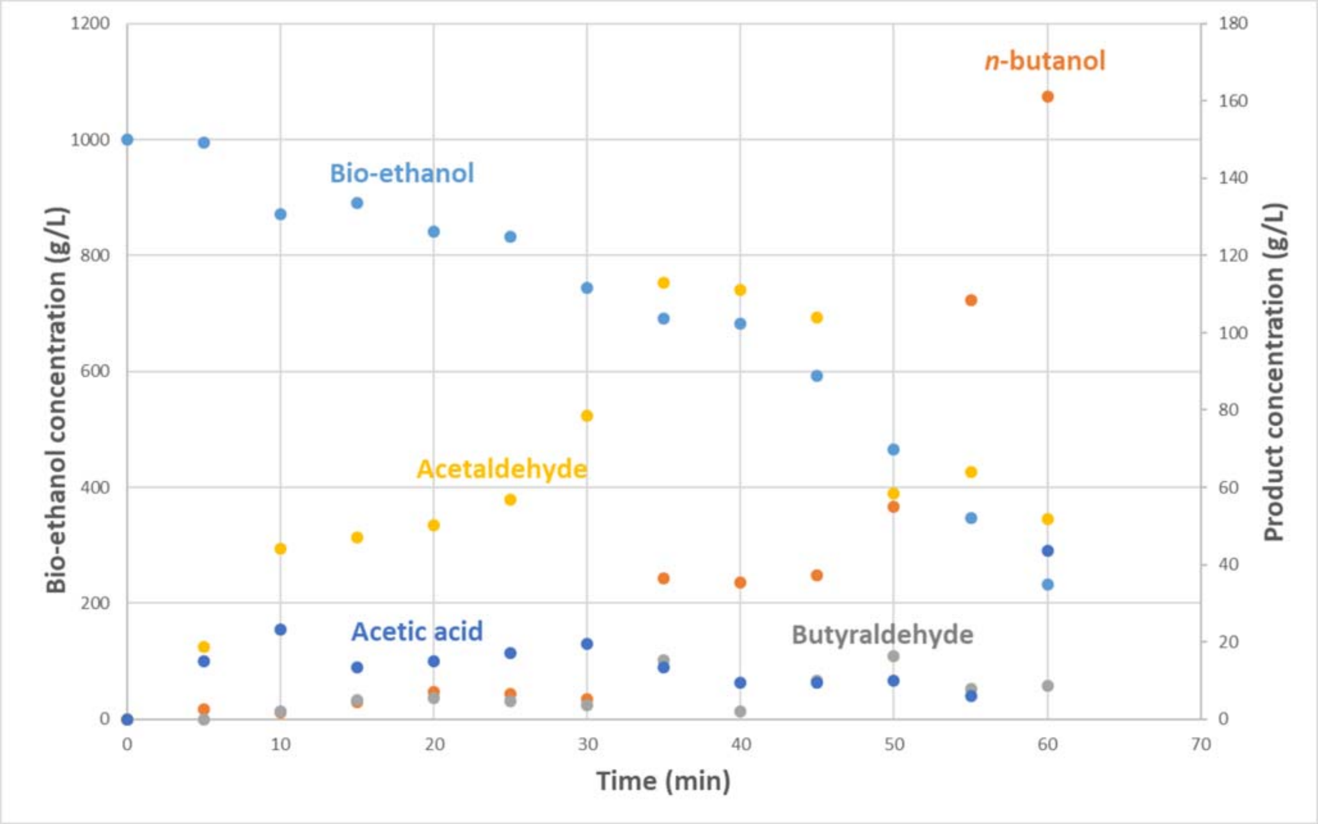


Figure 7: Effect of time at 250 °C on *n*-butanol-●, acetaldehyde-●, butyraldehyde-●, acetic acid-●, and bio-ethanol-● concentration

The by-product formation observed in this work is in line with other literature studies (Riittonen et al., 2012; Ghaziaskar and Xu, 2013; Dziugan et al., 2015). This encompasses previously reported work on conversion reactions with catalyst loading and temperature effect. However, the present research provides an inimitable phenomenon since this particular reaction has not been investigated using microwave heating before. The current conversion of bio-ethanol (76 wt.%) and *n*-butanol selectivity (60 %) in this study supersedes a variety of studies; Riittonen et al. (2012), obtained 25% conversion at 250 °C and 80% selectivity was achieved in 72 hrs, Jordison et al. (2016), achieved 46 % ethanol conversion at 230 °C in 10 hrs, and Yang et al. (2014), managed to obtain 15% conversion with 45% selectivity to *n*-butanol and 12% selectivity to C_6 alcohols.

Selectivity of the Ni-Al catalyst was initially directed toward acetaldehyde formation, with *n*-butanol concentration increasing gradually. The formation of intermediates/by-products such as acetaldehyde, butyraldehyde and acetic acid also occurred.

According to Chakraborty et al. (2015) and Tseng et al. (2016), acetaldehyde emerges from ethanol dehydrogenation and acetic acid forms via the oxidation of ethanol.

4.3.3.1.2 Bio-ethanol conversion

All the reactions were conducted under inert conditions by first purging each vessel with nitrogen gas (99.9%) followed by nitrogen loading at 10 bar in each vessel. After heating the microwave to 250 ºC, the pressure gradually increased as reaction time was increased. Reaction at 5 min reached 22.6 bar, whereas 60 min reaction reached 80.8 bar. The pressure remained virtually constant at 80.8 bar due to the pressure limit of the microwave. The gas-phase analysis could not be conducted due to the design of the microwave.

An overall plot is shown in Figure 8, which shows bio-ethanol conversion and *n*-butanol yield. As *n*-butanol yield increased, bio-ethanol conversion increased as well, with time (see Figure 8). An analysis of samples taken from 0 to 25 min showed increase with an increase in time leading to an increase in *n*-butanol formation from 0.10 to 0.22 g/g after 60 min. The total bio-ethanol conversion after 60 min was 76 wt.% with various product distribution presented in Section 3.3. The tested catalyst was investigated further for the effect of re-usability in producing *n*-butanol.

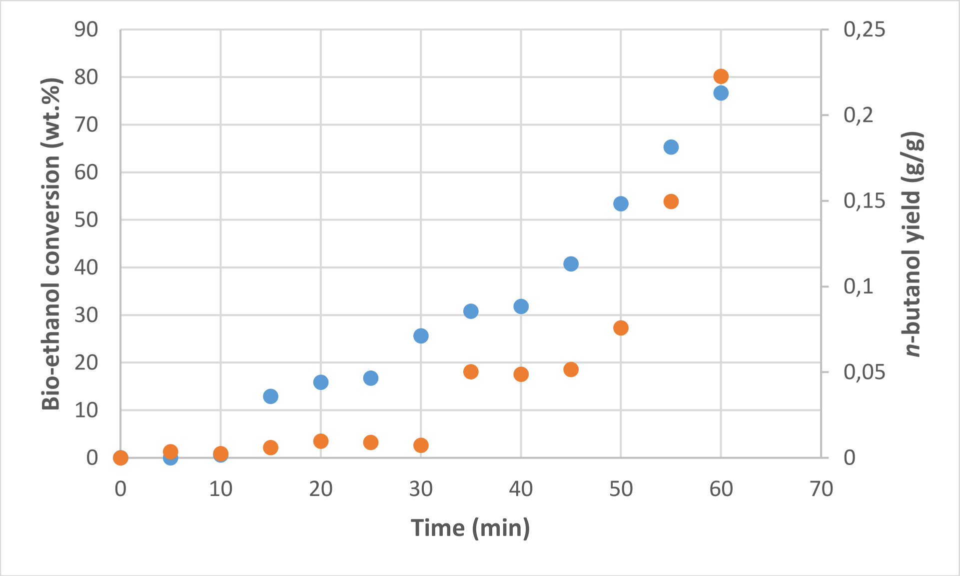


Figure 8: Effect of time on bio-ethanol conversion-(●) and *n*-butanol yield-(●)

4.3.3.1.3 The effect of Ni/Al_2O_3 catalyst reusability in producing n-butanol

Reusability of 5wt.% loading of Ni/Al_2O_3 catalyst was examined at 250 °C with a feed of bio-ethanol. Prior to each cycle of the experiment, the catalyst was separated by centrifugation, washed with deionised water, and then oven dried over night at 110 °C. The results obtained for ten successive reactions which were conducted in steps for 30 min each are summarized in Figure 9.

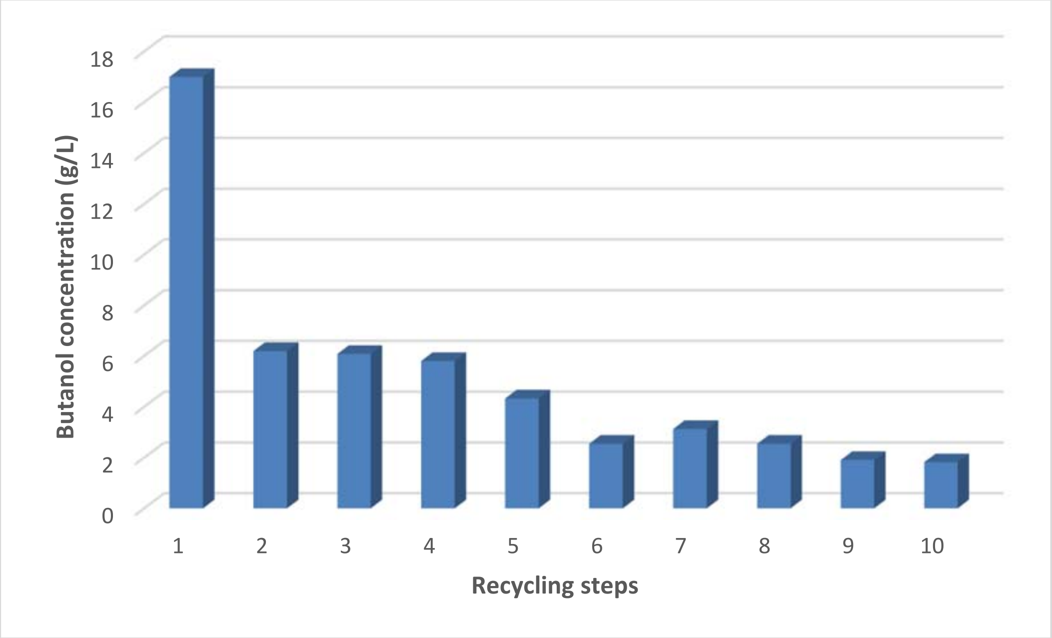


Figure 9 Effect of Ni/Al_2O_3 catalyst reusability on *n*-butanol concentration

The tested Ni/Al_2O_3 catalyst showed loss in catalytic activity in every consecutive run, and *n*-butanol concentration was reduced from 17 g/L to 2 g/L after the 10th run. According to Rane et al. (2016), this could be due to the leaching of nickel metal into the liquid. Another cause for butanol concentration decrease could be deactivation of the catalyst over time due to agglomeration or metal sintering. Other catalyst reusability studies in literature also report on severe drop in catalytic activity, which they also attribute to catalytic dissolution/ leaching (Leng et al., 2011; Mootabadi et al., 2012). The ability for recycling is always important for metal catalysed liquid-phase reactions. Similarly to Ji et al. (2009), it was found that the catalyst had been partially oxidised during reaction.

4.4 Conclusion

The main objective of this chapter was to develop a versatile method for maximal catalytic reaction conversion of bio-ethanol to *n*-butanol. The conducted experiments provided results indicating that microwave-assisted reaction for *n*-butanol production could be the most plausible reaction pathway compared to ABE process in Chapter 3. In this study, three different catalysts (MgO, Pt/Al_2O_3 and NiAl_2O_3) were investigated to produce *n*-butanol from bio-ethanol at different parameters. The experiments were formulated based on 2^3-level factorial design. From the results obtained, Ni/Al_2O_3 was selected to further investigate the reaction time at constant temperature and catalyst loading. This study successfully developed a rapid microwave heating method for bio-ethanol conversion to *n*-butanol in the presence of Ni/Al_2O_3 resulting in high *n*-butanol concentration of 161 g/L (0.22 g/g of bio-ethanol) at 250˚C within 60 min. The findings also proved that the choice of catalyst is also critical to achieve a high butanol yield during catalytic conversion reaction. This was corroborated by characterization results which proved Ni/Al_2O_3 to be the best catalyst compared to MgO and Pt/Al_2O_3. The microwave technology has a great potential in surpassing traditional catalytic conversion reaction as well as ABE process and serving as yet another step toward a wider portfolio of sustainable transportation fuels. Moreover, scaling-up chemical reactions and microwave-assisted catalyst synthesis in industries is of outmost importance. Once chemical reactions have been optimized in a small scale, and if the reaction products have proven to be useful, convenient and efficient production of large amounts becomes an issue. However, Anton Paar (Supplier of the microwave used in the study), is currently establishing direct method transfer from small-scale to industrial scale process by multiplying the amounts of the mixture and following the pre-optimized protocols from the small-scale methods. Therefore, this could lead to a great potential for large scale applications of the microwave assisted method for butanol production. Overall, the microwave system used in the current study revealed an inert, fast and convenient process for the catalytic conversion of bio-ethanol to *n*-butanol. This system can go a long way towards cost effectiveness of *n*-butanol production from bio-ethanol.

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CHAPTER 5

OVERALL CONCLUSION AND RECOMMENDATIONS

Overview

This chapter presents the conclusions based on the biochemical and chemical studies conducted. A set of experiments were conducted on bio-chemical and chemical butanol production, whereby the objectives were to produce butanol and organic acids from sweet sorghum juice using C. acetobutylicum and C. tetanomorphum as fermentation bacteria. This was done to create a traditional baseline for the thesis delivering an inimitable combination of clostridium species. The second objective of the study was to investigate microwave-assisted catalytic reactions for the production of n-butanol from bio-ethanol using metal oxide as catalysts. Science contribution of the study and advise for future work is also presented in this chapter.

5.1 CONCLUSIONS

The study carefully reviewed the bio-butanol production technologies to fully provide a new generation energy that can offer many attractive features for transportation fuel. The pioneered research on ABE process has encountered numerous limitations. Given this, various studies are being undertaken to improve the butanol fermentation process. These include the use of *Clostridium* genetic manipulation and feedstock investigation, which can lower fermentation constraints. This study successfully investigated ABE process for butanol production from sweet sorghum juice. Bio-chemical reactions using *C. acetobutylicum* resulted in the highest butanol concentration of 6.49 g/L (0.06g/g of sweet sorghum juice) after 96 hrs of fermentation when using an inoculum loading of 10% v/v (OD of 0.81). Whereas butanol concentration of 2.66 g/L was obtained at 5% v/v *C. tetanomorphum*, with 10% v/v *C. tetanomorphum* producing the highest product (butyric acid) of 5 g/L. The results presented an assertion of *C. acetobutylicum* as the best bacteria for butanol production from sweet sorghum juice compared to *C. tetanomorphum*. The research in this area can be further explored to encompass numerous factors to circumvent the encountered challenges of elongated fermentation time and low concentrations.

To avoid elongated time from the ABE process, a microwave-assisted system for catalytic reactions using metal oxide catalysts (MgO, Pt/Al_2O_3 and Ni/Al_2O_3) was employed. The microwave reaction successfully presented that more butanol could be produced in a shorter time in comparison to the ABE route, which produced bio-based butanol concentration 6.49 g/L (0.06 g/g of sweet sorghum juice) in 96 hrs of fermentation. The presented data generated from this study demonstrate a higher *n*-butanol concentration of 161 g/L (0.22g/g of bio-ethanol) when 5wt% Ni/Al_2O_3 catalyst loading was used, in 60 min reaction time. Indeed, this confirms the execution of a rapid catalytic conversion of bio-ethanol to *n*-butanol using a microwave reactor. The rationale behind this technology can be further explored in the realisation of a sustainable energy path with a view towards the prodigious trajectory for butanol production.

The catalytic experiments demonstrated valuable results indicating that this could be the most feasible reaction pathway for producing butanol. The microwave technology alone offers advantageous factors, such as shorter reaction time, as well as less temperature input compared to ABE fermentation and conventional heating. This technology is applicable in many fields of research, but here is a big chance to highly compete with conventional bio-butanol processes. The future aspiration of utilising microwave systems must be aimed at investigating a variety of catalysts to further understand microwave technology's role for *n*-butanol formation. The microwave system revealed a fast and convenient process for the catalytic conversion of bio-ethanol to *n*-butanol. This can go a long way towards the sustainability of *n*-butanol production.

5.2 CONTRIBUTION TO SCIENCE

The following accomplishments made throughout this investigation are considered to be esteemed additions to the current biofuel scientific knowledge:

- Development of a method which can be used for long term storage of sweet sorghum juice
- Co-fermentation using *C. acetobutylicum* and *C. tetanomorphum* to produce high organic acid concentrations (Results presented in Appendix C)

- Bio-butanol production using *C. acetobutylicum* from a pre-stored sweet sorghum juice
- Development of a fast and convenient microwave-assisted method for producing butanol from bio-ethanol using metal oxide nano-catalysts.

In South Africa, sweet sorghum and sugarcane stalks are abundant renewable resources which have the potential for biofuel production. The industrial use of biologically derived ethanol to produce an even better alcohol (butanol) under microwave reactor would be a great accomplishment towards realization of a sustainable future for all. Anton-Paar microwave reactor used in the current study is in the process of being tested for large scale application, this could lead to metal oxide catalyst application for butanol production on a large scale production. Industrially, butanol is highly recommended as a great promise for petrol substitute. The prime reason for butanol production relies on its performance in comparison to current petrol and bio-ethanol. Therefore, with all the literature key points stressed in this study, showing the sustainability of considering bio-butanol as a fuel and the potential microwave-assisted technology for its production, it is vital to use this type of fuel in the near future, since market demand is expected to increase by 2020.

5.3 RECOMMENDATIONS

- Future work should look at using online continuous microwave systems in order to be able to measure the amount of gases formed during the reaction.
- Self-prepared catalysts using microwave synthesis for higher catalytic activity should be considered.
- Investigation and comparison of both power and temperature during the reaction should be considered.
- Amount variation of bio-ethanol feed should also be considered for *n*-butanol production.
- More research should be initiated to manipulate the current techniques for large-scale butanol production.

- Overall, microwave-assisted technology with the use of metal oxides as catalysts for *n*-butanol production might contribute to a significant platform for future development.

APPENDIX A

STANDARD CALIBRATION CURVES

Prior to High Performance Liquid Chromatography analysis, calibration curves for sugars, acids, and alcohols were conducted. The concentrations of the stock solutions were serially diluted and from 5-0.313g/L equivalent to 0.5g/100mL for each stock solution. Thereafter, a straight line was fitted to the data and a constant (k) was obtained which was used in the concentration calculations for each composition in the samples.

Table A1 Calibration standards and their actual mass weighed

Standards	**Actual mass (g/100mL)**
Sucrose	0.502
Xylose	0.505
Fructose	0.518
Glucose	0.512
1-Butanol/*n*-Butanol	0.519
2-Butanol	0.517
Acetic acid	0.516
Butyric acid	0.520
Ethyl acetate	0.501
Acetaldehyde	0.539
Butyraldehyde	0.518
Succinic acid	0.515
Lactic acid	0.510

Table A2 Retention times for calibration standards

Standards	**Retention times (min)**
Sucrose	10.9-11.1
Fructose	15.1-16
Glucose	12.4-12.7
1-Butanol	36-38
2-Butanol	30-31
Acetic acid	14-14.8
Butyric acid	21-22
Ethyl acetate	28-28.7
Acetaldehyde	17-18
Butyraldehyde	29-29.4
Succinic acid	12.1-12.5
Lactic acid	19-19.5

APPENDIX A1

STANDARD CALIBRATION CURVES FOR CHAPTER 3

Data from chapter 3 was calculated based on the calibrations standards from Figure A1.1-A1.10 and table A3.

Table A3 Peak areas obtained from the HPLC for each calibration standard

Concentration (g/L)	Sucrose	Fructose	Glucose	1-Butanol	Acetic acid	Butyric acid	Succinic acid	Acetone	Ethanol	Lactic acid
10	1.74E+05	6.89E+05	7.15E+05	4.66E+05	3.16E+05		3.63E+05	2.83E+05	2.98E+05	3.07E+05
5	2.35E+05	3.43E+05	3.53E+05	2.34E+05	1.56E+05	2.26E+05	2.30E+05	1.51E+05	1.47E+05	1.52E+05
2.5	1.13E+05	1.71E+05	1.76E+05	1.22E+05	7.87E+04	1.12E+05	1.14E+05	6.77E+04	6.81E+04	7.38E+04
1.25	5.60E+04	8.52E+04	8.77E+04	5.95E+04	4.09E+04	5.05E+04	5.25E+04	3.40E+04	3.34E+04	3.63E+04
0.625	2.78E+04	4.09E+04	4.71E+04	3.12E+04	2.13E+04	2.00E+04	2.63E+04	1.97E+04	1.64E+04	1.87E+04
0.313	1.50E+04	2.02E+04	2.68E+04	1.37E+04	1.12E+04	1.10E+04	1.31E+04	9.85E+03	8.18E+03	8.29E+03

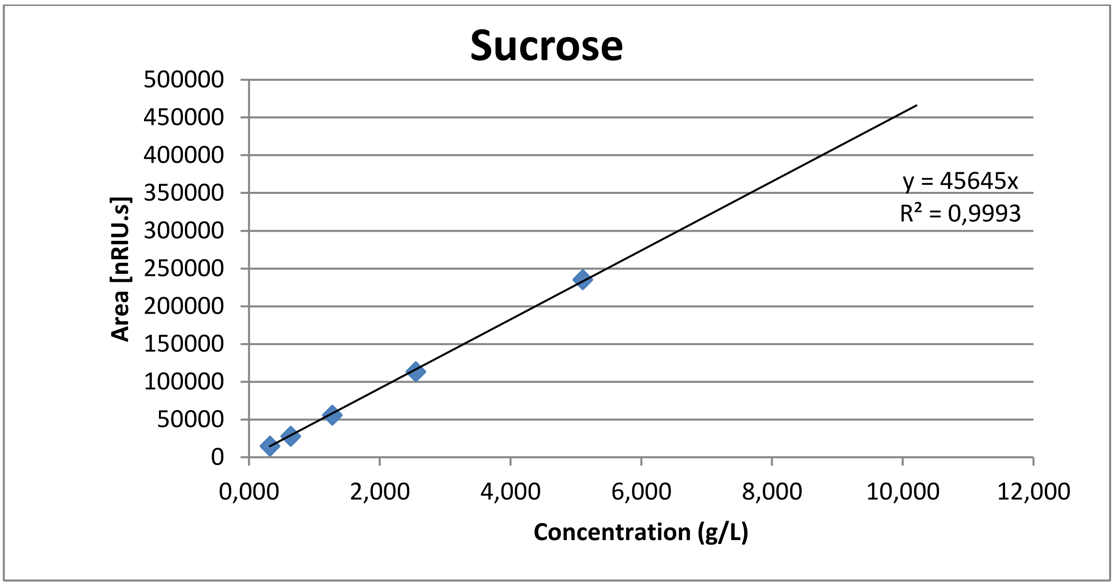


Figure A1.1 Sucrose calibration curve

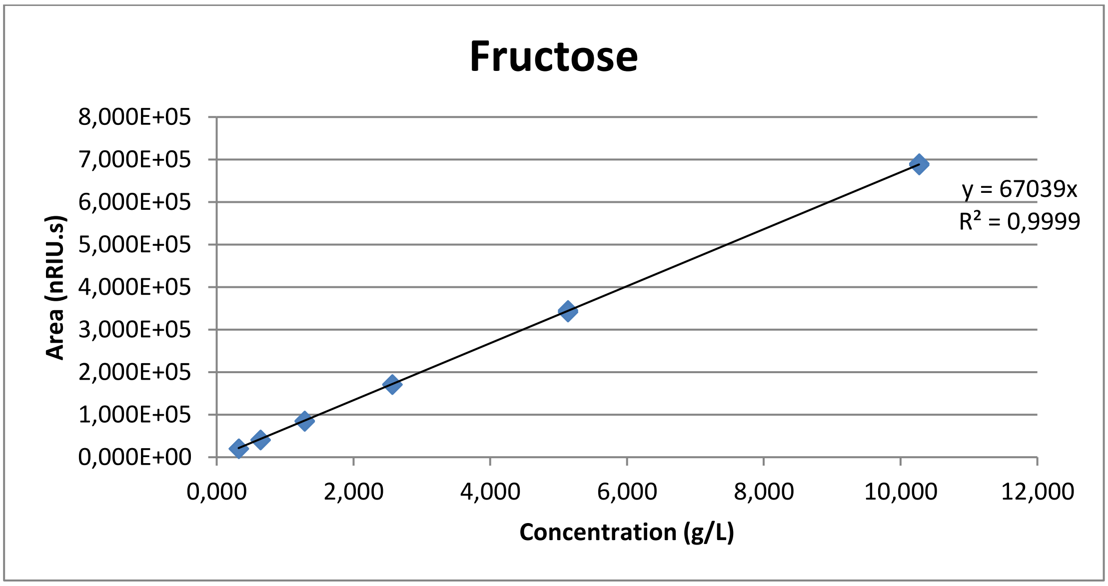


Figure A1.2 Fructose calibration curve

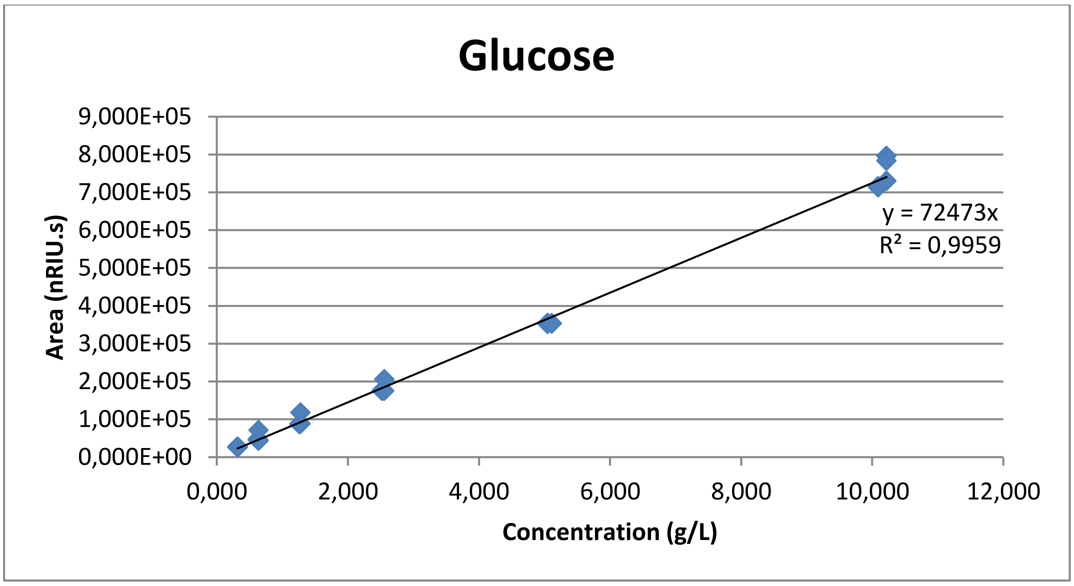


Figure A1.3 Glucose calibration curve

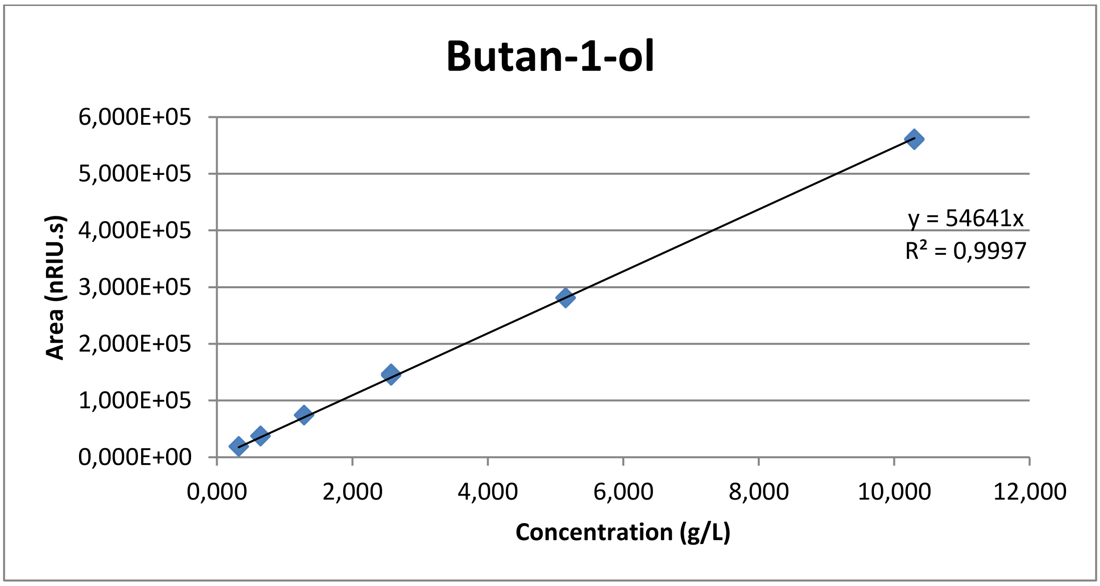


Figure A1.4 Butan-1-ol calibration curve

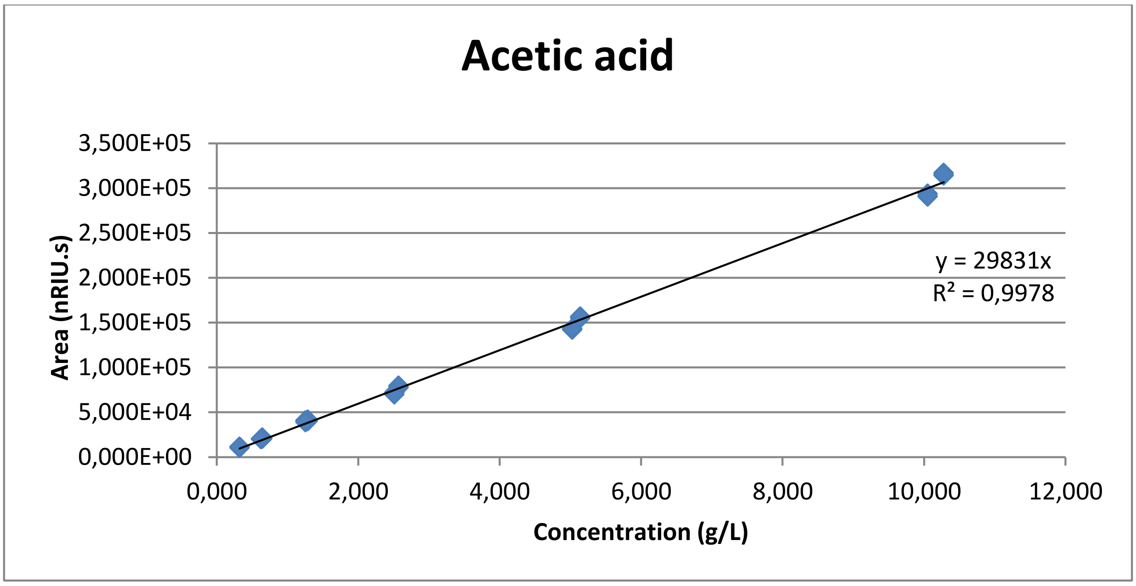


Figure A1.5 Acetic acid calibration curve

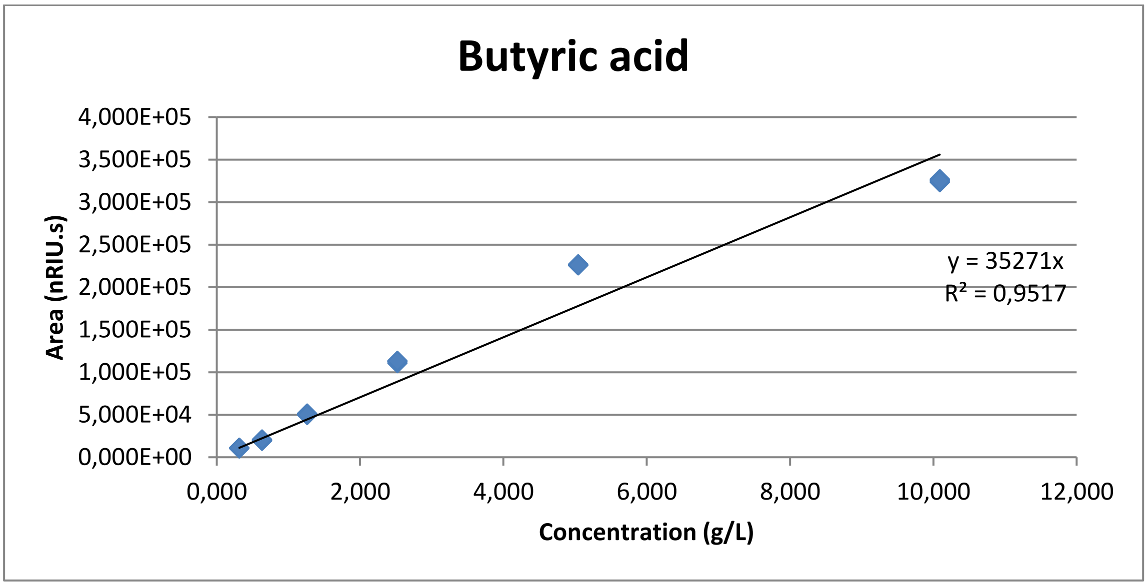


Figure A1.6 Butyric acid calibration curve

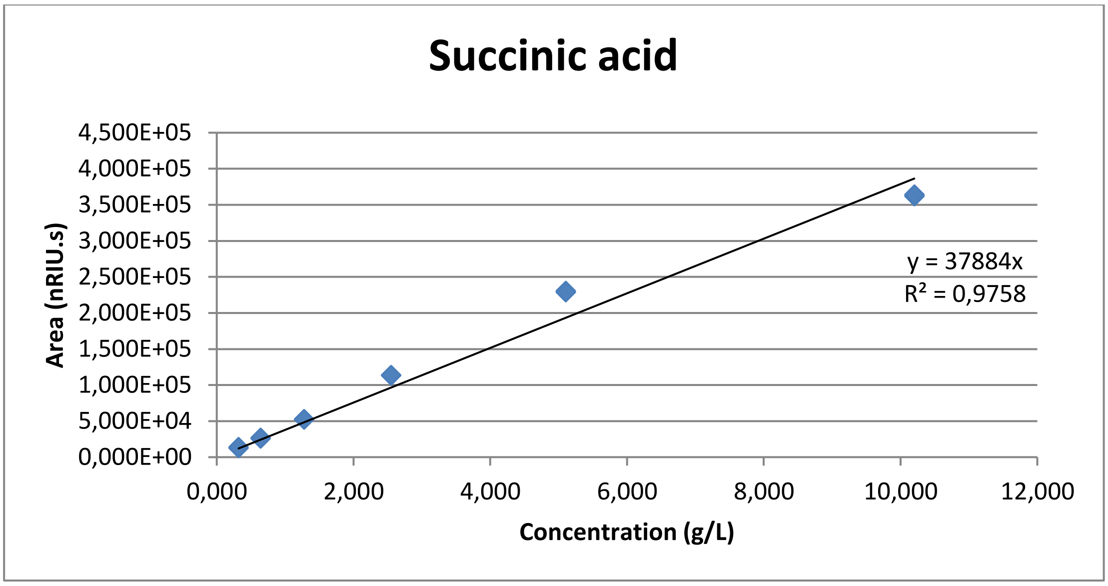


Figure A1.7 Succinic acid calibration curve

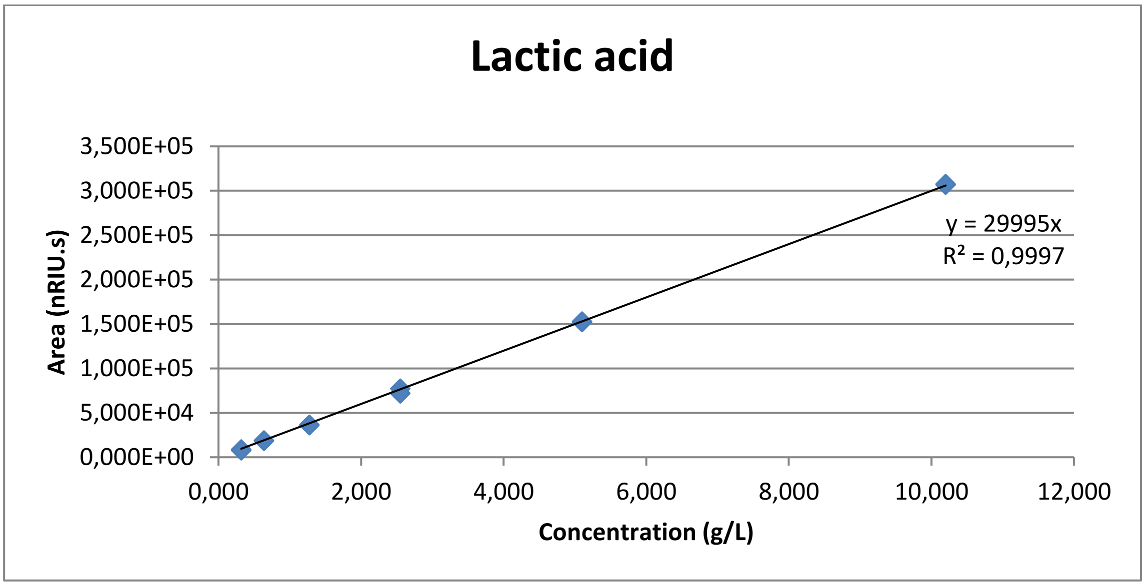


Figure A1.8 Lactic acid calibration curve

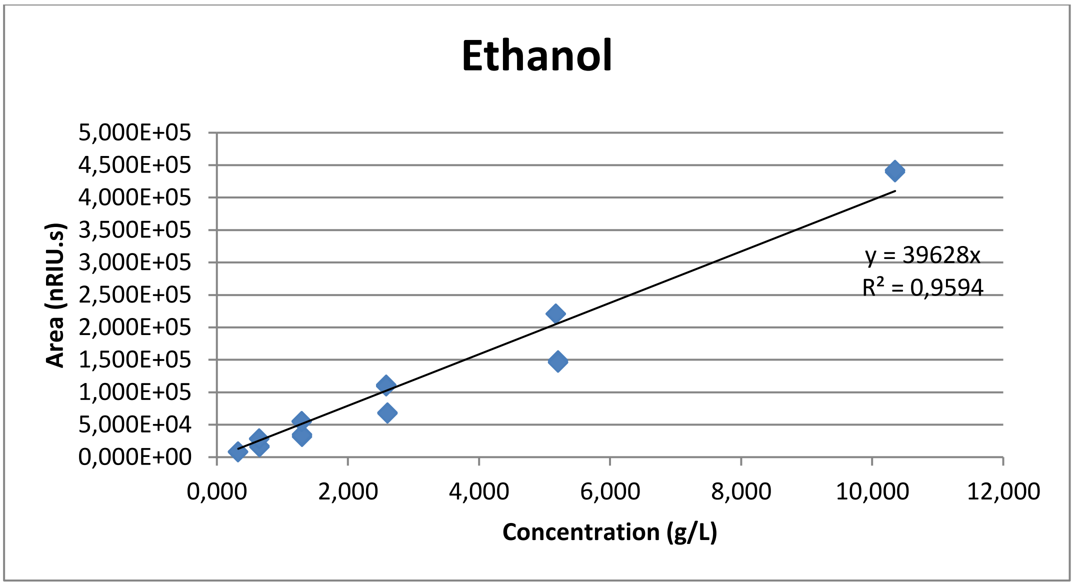


Figure A1.9 Ethanol calibration curve

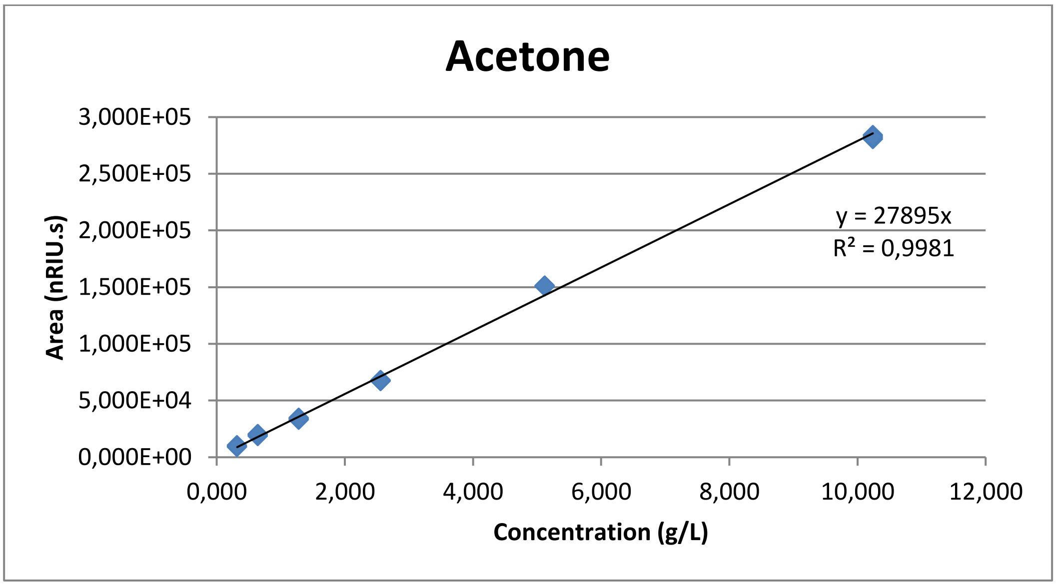


Figure A1.10 Acetone calibration curve

APPENDIX A2

STANDARD CALIBRATION CURVES FOR CHAPTER 4

Data from chapter 4 was calculated based on the calibrations standards from Figure A2.1- and Table A4.

Table A4 Peak areas obtained from the HPLC for each calibration standard

Concentration (g/L)	Ethanol	Acetaldehyde	Butyraldehyde	Butan-1-ol	Acetic acid
5	3.021E+05	3.334E+05	4.326E+05	4.205E+05	3.182E+05
2.5	1.462E+05	1.621E+05	2.020E+05	2.151E+05	1.565E+05
1.25		7.977E+04	9.881E+04	1.209E+05	8.612E+04
0.625		3.808E+04	4.761E+04	5.663E+04	4.689E+04
0.313	1.961E+04	1.968E+04	2.410E+04	2.880E+04	2.501E+04

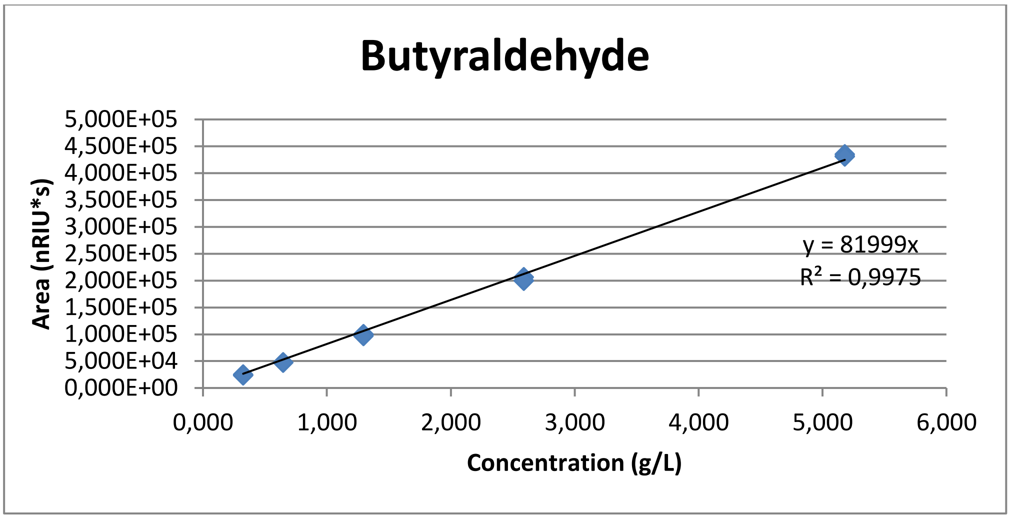


Figure A2.1Butyraldehyde calibration curve

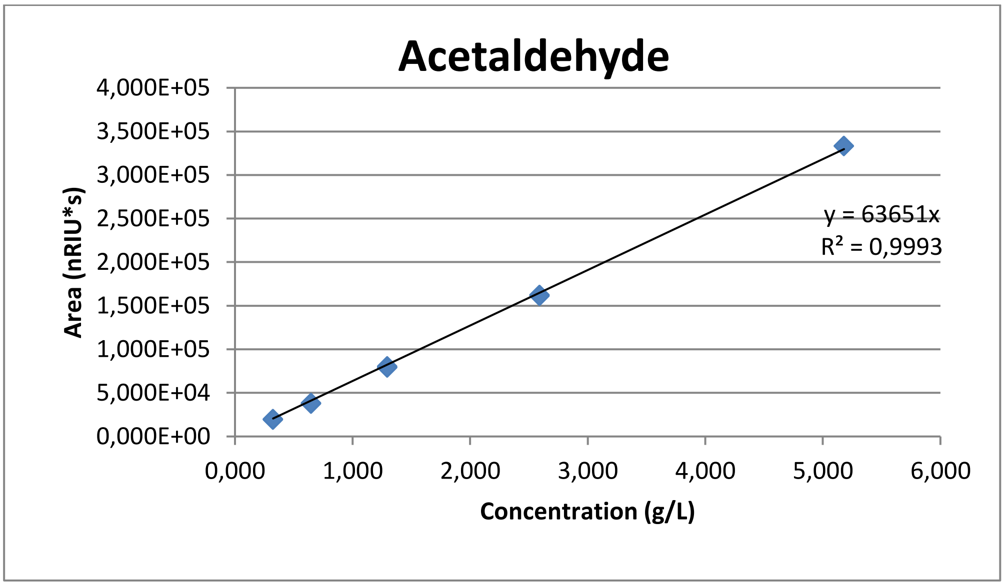


Figure A2.2 Acetaldehyde calibration curve

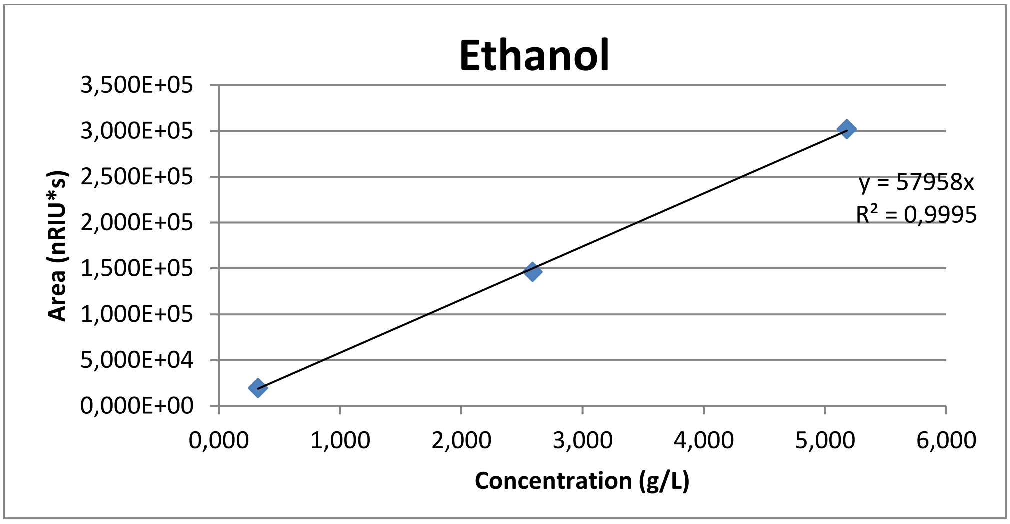


Figure A2.3 Ethanol calibration curve

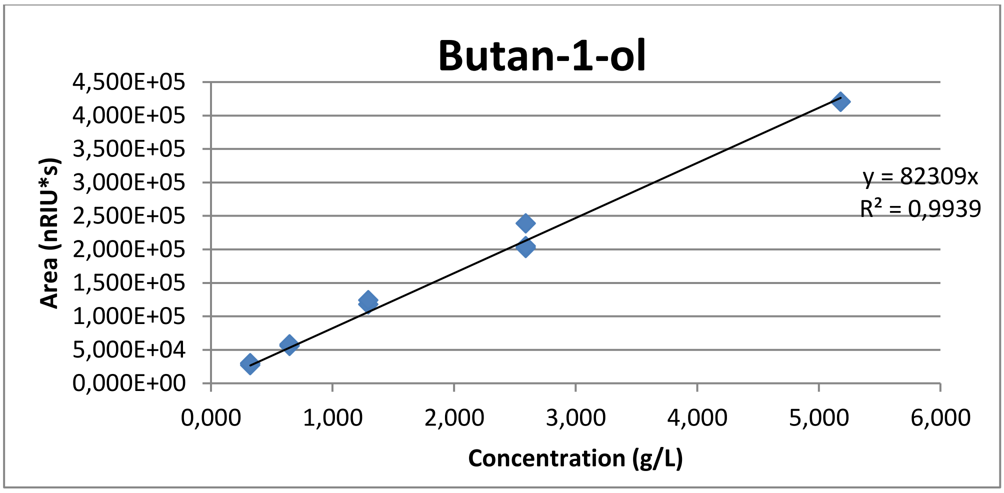


Figure A2.4 Butan-1-ol calibration curve

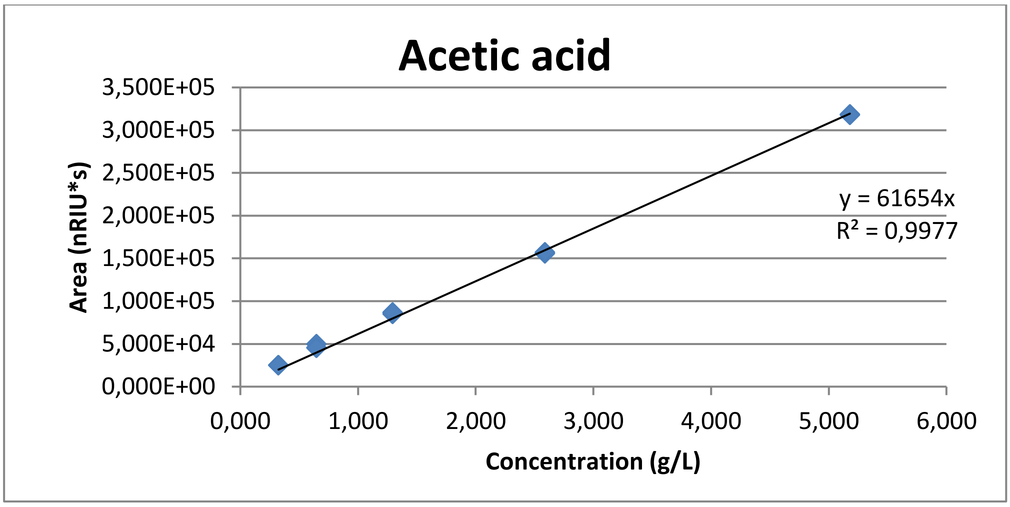


Figure A2.5 Acetic acid calibration curve

APPENDIX B

EXPERIMENTAL ERROR AND CALCULATIONS

This Section shows the experimental errors associated with the parameters investigated in chapter 3 of the current study.

The experimental error was calculated and determined using Equation B.1

(Mutepe, 2011)

$$\% \mathbf{Error} = \frac{\text{Confidence limit}}{\bar{\bar{\text{X}}}} \text{X } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{B.1}$$

Where x is the average

Confidence limit is calculated using the following equation:

$$\bar{x} \pm 1.96\left(\frac{\sigma}{\sqrt{n}}\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{B. 2}$$

The standard deviation must first be determined in order to calculate 95% confidence limit; The standard deviation is calculated as follows:

$$\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{n-1}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{B.3}$$

Where x is the sample mean, $\bar{x}$ is the average and n is the sample size.

Calculations for sugar concentrations in the biomass

$$\boldsymbol{C}_{s(\frac{g}{L})} = \frac{\text{Area of sugar}}{\text{Slope of sugar calibration}} \text{X dilutiona factor} \ldots\ldots\ldots\ldots\ldots\ldots 1$$

C$_{\mathbf{s}}$ (Concentration of sugars) was used in equation 2

$$\boldsymbol{C}_{s(\frac{g}{g})} = \frac{\text{Cs x Total volume (L)}}{\text{wt of biomass}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 2$$

The same Equations were also used for ethanol calculations

APPENDIX C

ADDITIONAL RESULTS FOR CHAPTER 3

This section shows additional outputs from *Clostridium* co-culture fermentation at different time intervals (12, 54 and 96 hrs). These results were not shown on the chapter 3 results, only single culture fermentation results were shown.

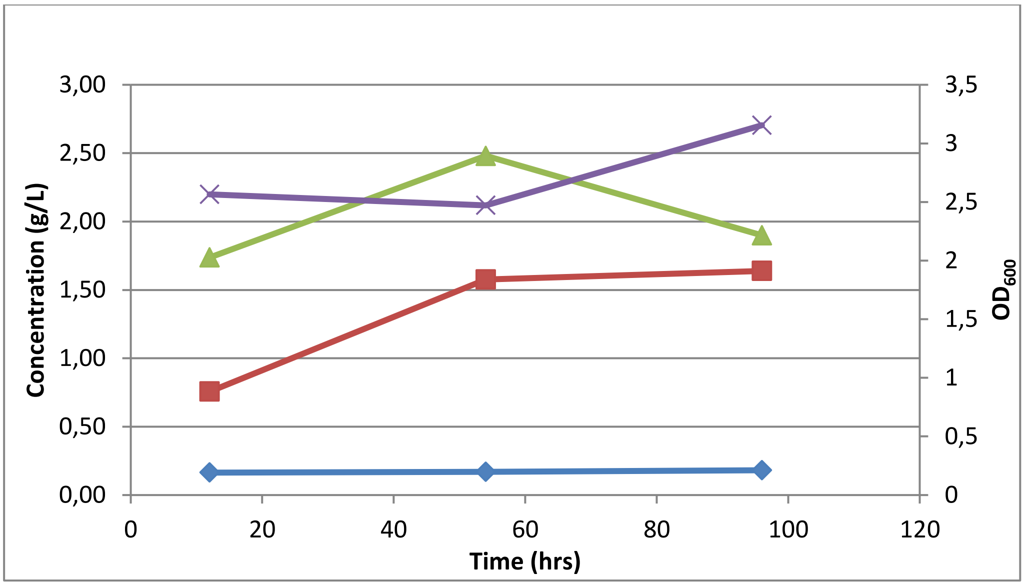


Figure C1 3:3 *C. acetobutylicum to C. tetanomorphum* (♦Acetic acid, ■ succinic acid, ▲ Lactic acid × Cell growth.

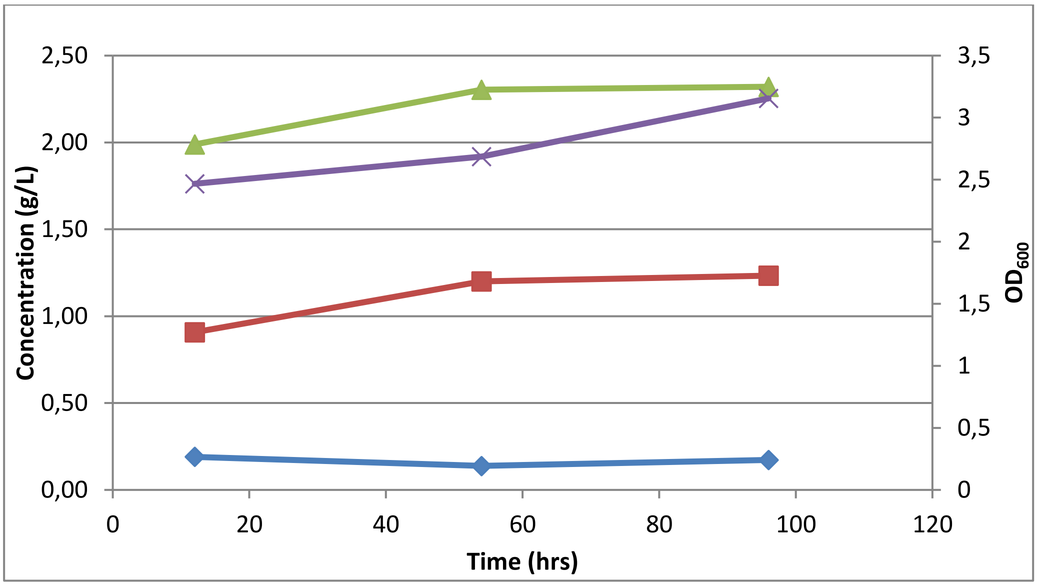


Figure C2 3:10 *C. acetobutylicum to C. tetanomorphum* (♦Acetic acid, ■ succinic acid, ▲ Lactic acid × Cell growth.

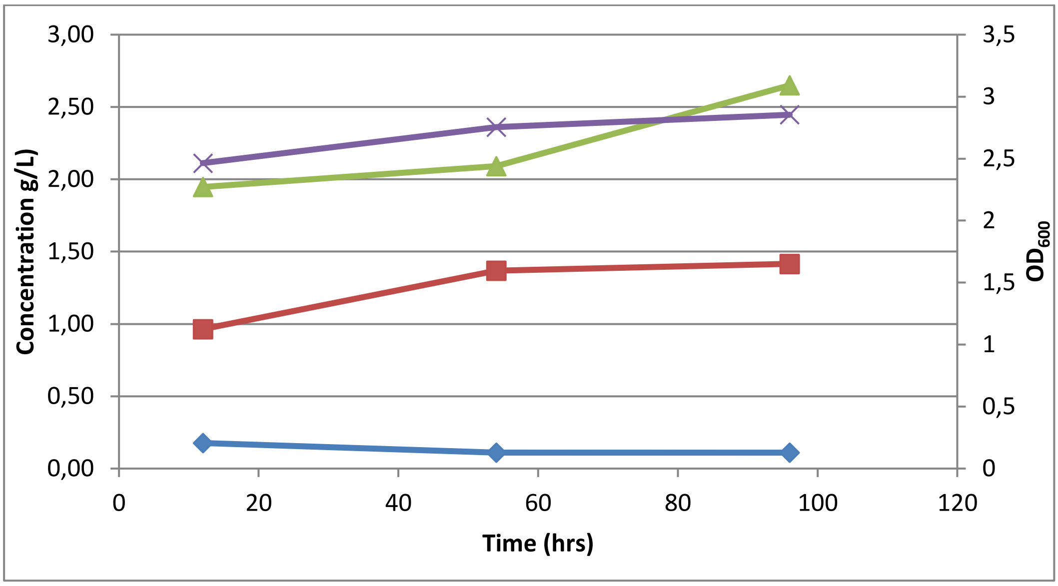


Figure C3 10:3 *C. acetobutylicum to C. tetanomorphum* (♦Acetic acid, ■ succinic acid, ▲ Lactic acid × Cell growth.

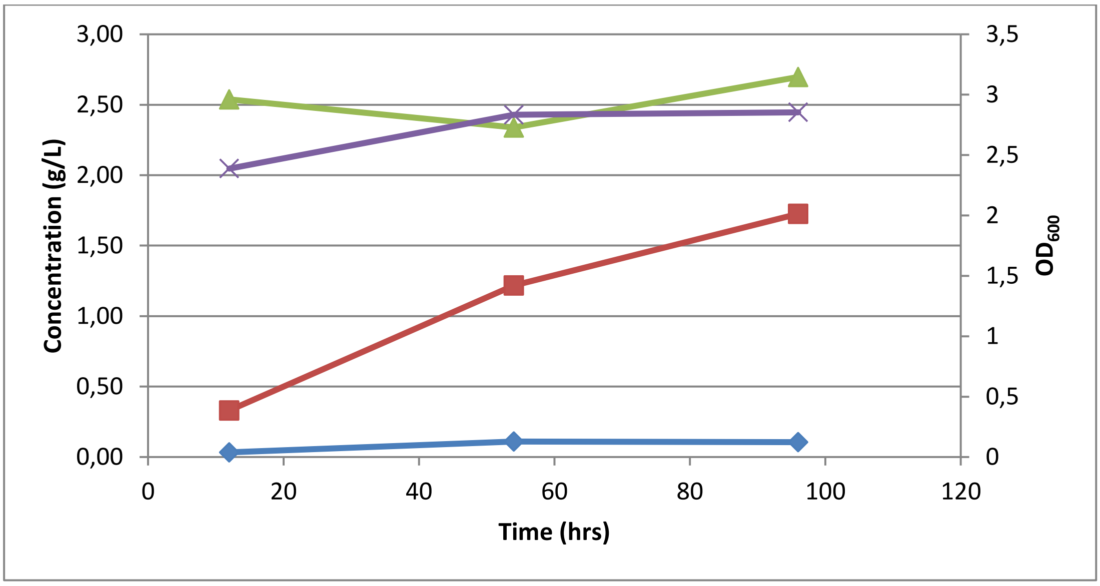


Figure C4 6.5:6.5 *C. acetobutylicum to C. tetanomorphum* (♦Acetic acid, ■ succinic acid, ▲ Lactic acid × Cell growth.

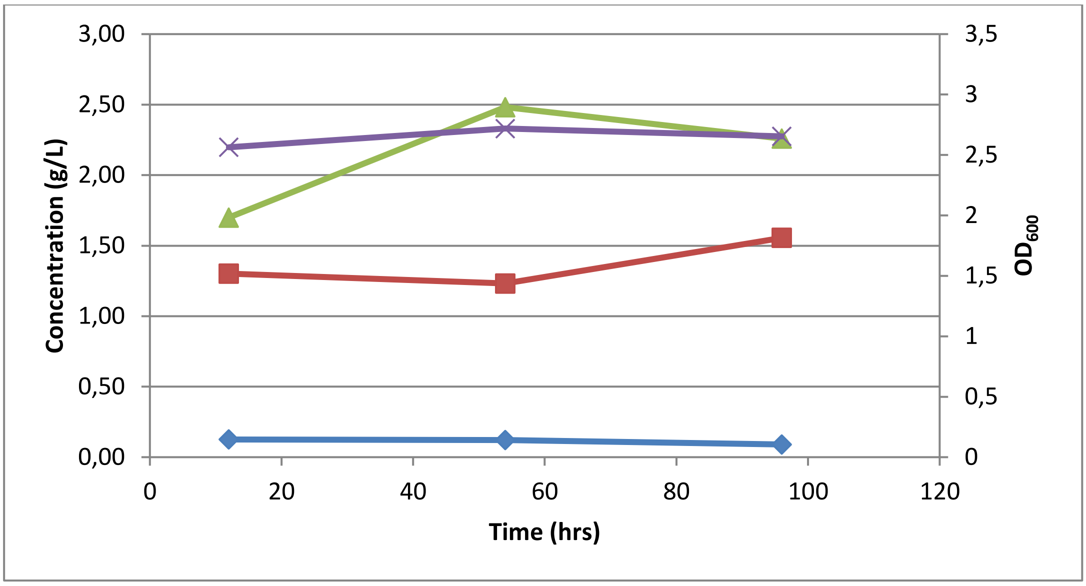


Figure C5 10:10 *C. acetobutylicum to C. tetanomorphum* (◆Acetic acid, ■ succinic acid, ▲ Lactic acid × Cell growth.

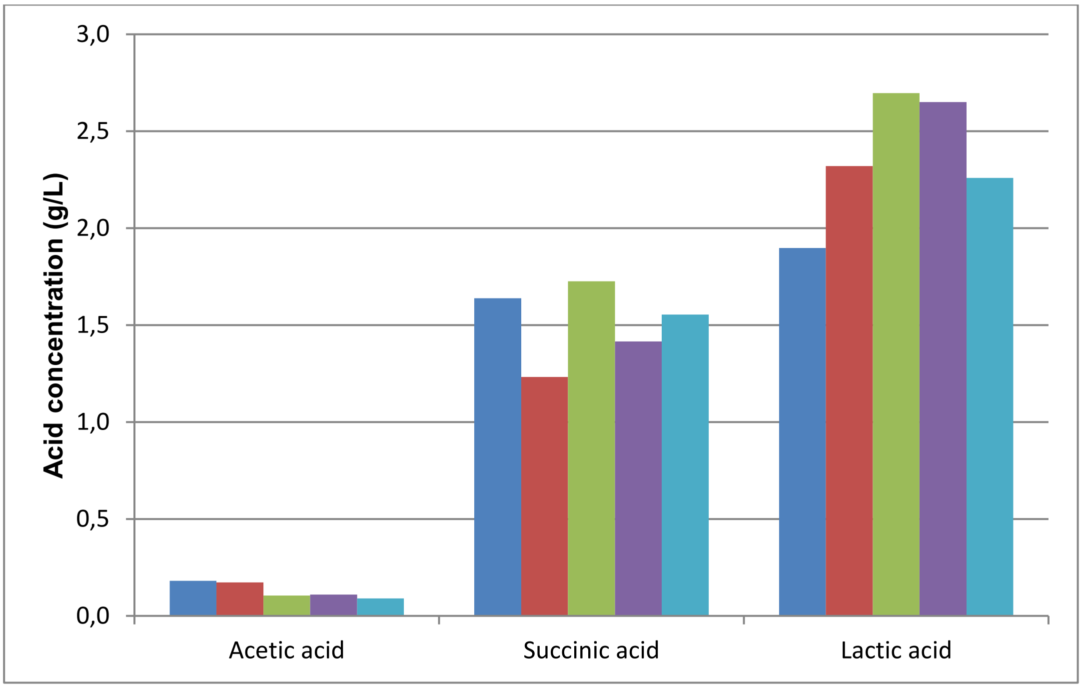


Figure C6 Acid concentrations (g/L) obtained for different inoculum combinations (■-3:3, ■-3:10, ■-6.5:6.5, ■-10;3, ■-10:10 %v/v) of *C.acetobulycum* to *C. tetanomorphum*

APPENDIX D

ADDITIONAL RESULTS FOR CHAPTER 4

In this study, 3 different catalysts were tested at lowest and highest percentage loading (1 and 5wt %) and temperature (200 and 250˚C). These optimizations were conducted to obtain the highest *n*-butanol producing catalyst.

This section represents additional results (by-product yield) from other catalysts which were not part of Chapter 4. Results for catalysts weight loss are also shown in Figure D4. Formula for calculation *n*-butanol yield, selectivity (%), and bio-ethanol conversion (%) are shown here.

$$\boldsymbol{Yield} = \frac{\text{wt of the product}}{\text{wt of EtOH}}$$

$$\boldsymbol{EtOH\ conversion}\ (\%) = \frac{1 - \text{wt of EtOH}}{\text{wt of products} + \text{wt of EtOH}}\ \text{X}\ 100\%$$

$$\boldsymbol{Selectivity}\ (\%) = \frac{\%\text{Product yield}}{\%\text{EtOH conversion}}\,\text{x}100\%$$

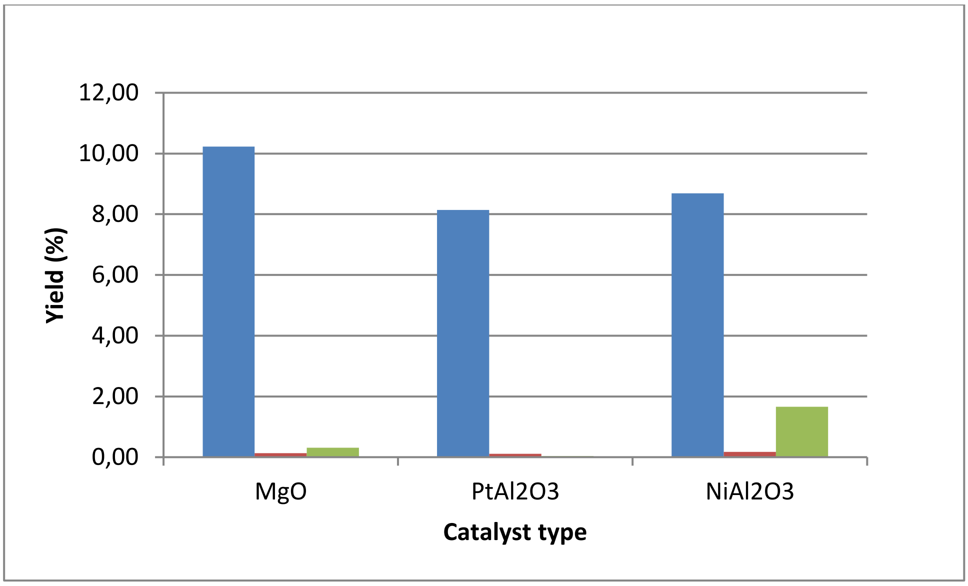


Figure D1 Effect of 1 wt % catalyst loading on acetaldehyde (🟦), butyraldehyde (🟥) and *n*-butanol (🟩) yield at 200°C

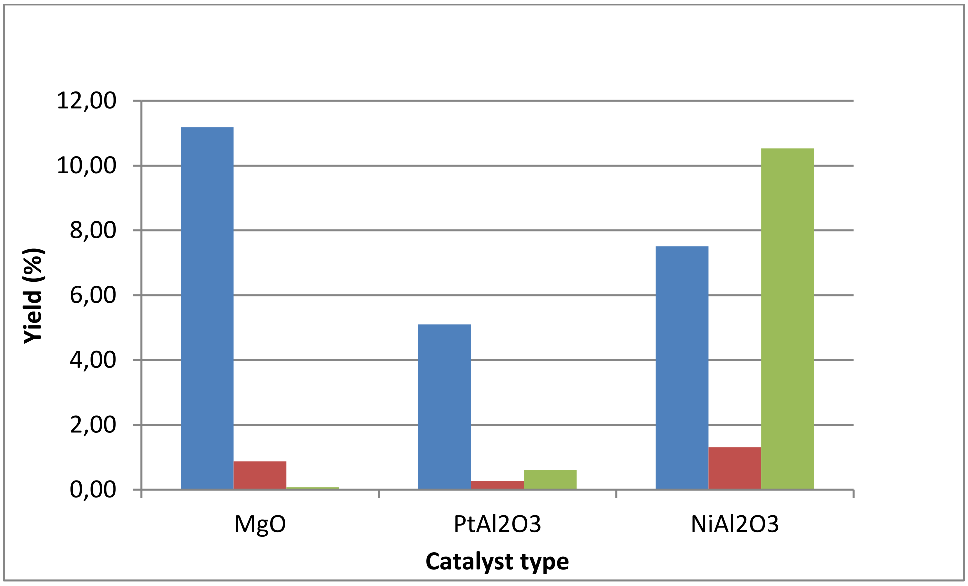


Figure D2 Effect of 5 wt % catalyst loading on acetaldehyde (🟦), butyraldehyde (🟥) and *n*-butanol (🟩) yield at 200°C

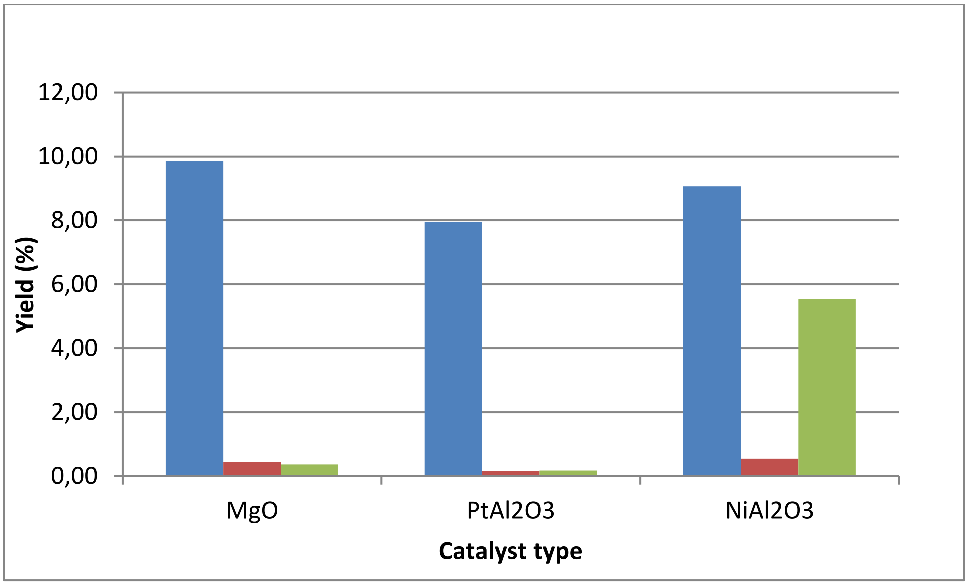


Figure D3 Effect of 1 wt % catalyst loading on acetaldehyde (🟦), butyraldehyde (🟥) and *n*-butanol (🟩) yield at 250°C

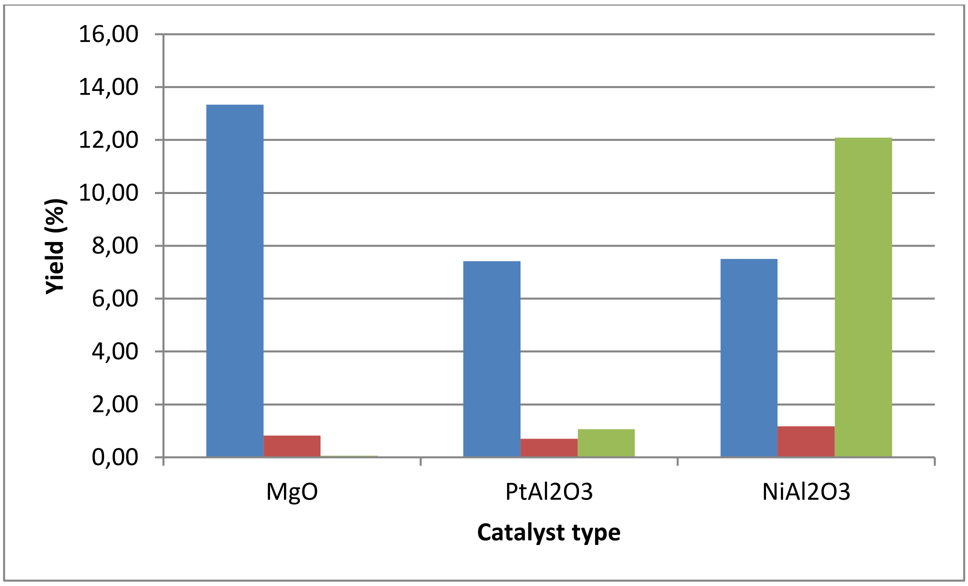


Figure D4 Effect of 5 wt % catalyst loading on acetaldehyde (🟦), butyraldehyde (🟥) and *n*-butanol (🟩) yield at 250°C

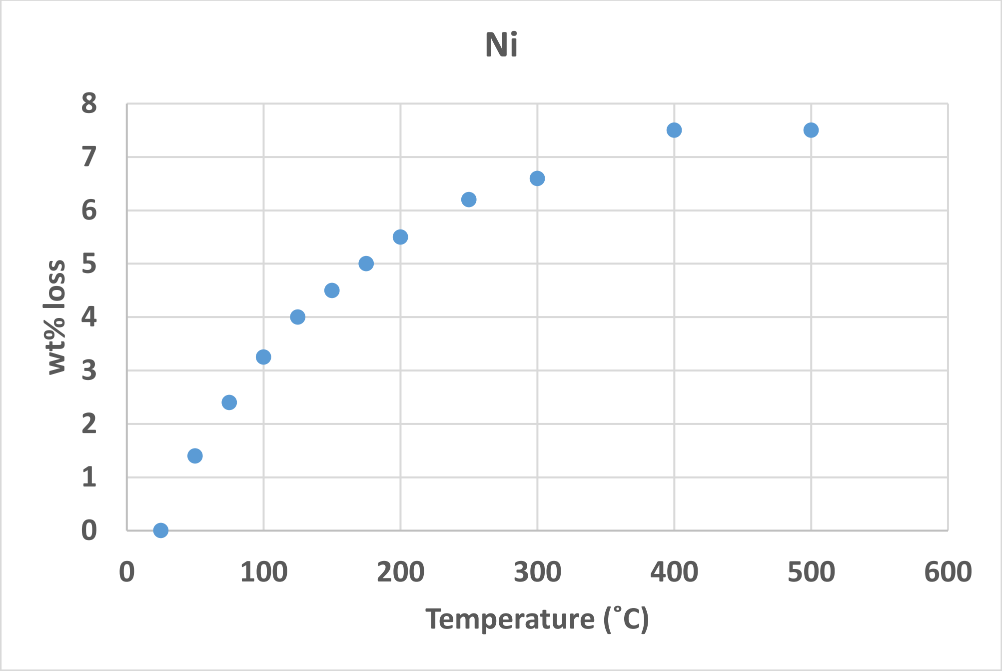


Figure D5 Weight loss during Ni/Al_2O_3TGA analysis

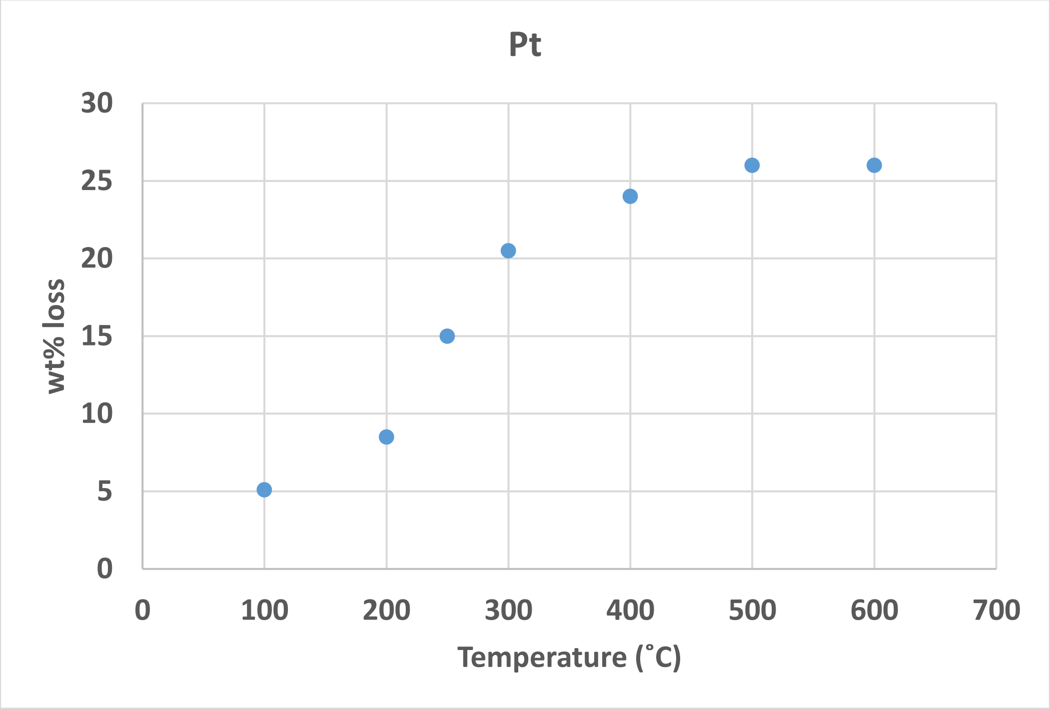


Figure D6 Weight loss during Pt/Al_2O_3TGA analysis

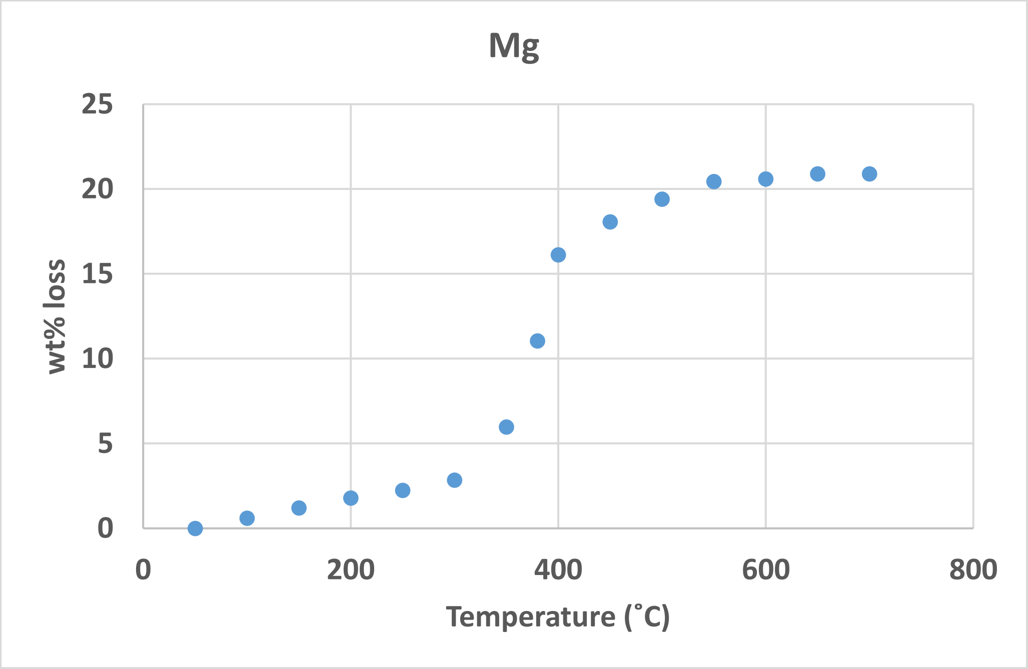


Figure D7 Weight loss during MgO TGA analysis

Material Safety Data Sheet
ETHANOL

Section 1 - Chemical Product and Company Identification

MSDS Name: ETHANOL
Synonyms: Ethyl alcohol; Ethyl hydroxide; Fermentation alcohol; Grain alcohol; Methylcarbinol, Ethanol absolute 99.9%, Ethanol rectified 96%.
Company Identification:
ROCHELLE CHEMICALS & LAB EQUIPMENT cc
54 Meson Road, Electron, Johannesburg, South Africa
For information, call: +27 11 613 5638
Emergency Number: +27 83 269 7693

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
64-17-5	Ethyl alcohol	95-99.9	200-578-6
7732-18-5	Water	1-5	231-791-2

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: colorless clear liquid. Flash Point: 16.6 deg C.
Warning! Causes severe eye irritation. **Flammable liquid and vapor.** Causes respiratory tract irritation. This substance has caused adverse reproductive and fetal effects in humans. May cause central nervous system depression. May cause liver, kidney and heart damage. Causes moderate skin irritation.
Target Organs: Kidneys, heart, central nervous system, liver.

SIGMA-ALDRICH

sigma-aldrich.com

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.1 Revision Date 10.08.2016
Print Date 02.08.2018
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1 Product identifiers

Product name : Magnesium oxide

Product Number : 529699
Brand : Aldrich
REACH No. : A registration number is not available for this substance as the substance or its uses are exempted from registration, the annual tonnage does not require a registration or the registration is envisaged for a later registration deadline.
CAS-No. : 1309-48-4

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich (Pty.) Ltd.
17 Pomona Street
Aviation Park, Unit 4
KEMPTON PARK
1619 SOUTH AFRICA

Telephone : +27 11 979 1188
Fax : +27 11 979 1119

1.4 Emergency telephone number

Emergency Phone #

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.2 Label elements

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.3 Other hazards

This substance/mixture contains no components considered to be either persistent, bioaccumulative and toxic (PBT), or very persistent and very bioaccumulative (vPvB) at levels of 0.1% or higher.

SECTION 3: Composition/information on ingredients

3.1 Substances

Formula : MgO
Molecular weight : 40,3 g/mol
CAS-No. : 1309-48-4
EC-No. : 215-171-9

SAFETY DATA SHEET

according to Regulation (EC) No. 1907/2006
Version 5.0 Revision Date 12.12.2012
Print Date 02.08.2018
GENERIC EU MSDS - NO COUNTRY SPECIFIC DATA - NO OEL DATA

1. IDENTIFICATION OF THE SUBSTANCE/MIXTURE AND OF THE COMPANY/UNDERTAKING

1.1 Product identifiers

Product name	:	Platinum on alumina
Product Number	:	311324
Brand	:	Aldrich

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich (Pty.) Ltd.
17 Pomona Street
Aviation Park, Unit 4
KEMPTON PARK
1619 SOUTH AFRICA

Telephone : +27 11 979 1188
Fax : +27 11 979 1119

1.4 Emergency telephone number

Emergency Phone # :

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.
Not a hazardous substance or mixture according to EC-directives 67/548/EEC or 1999/45/EC.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008 [CLP]

Pictogram	none
Signal word	none
Hazard statement(s)	none
Precautionary statement(s)	none
Supplemental Hazard Statements	none

Safety data sheet available on request.

Safety data sheet available on request for professional users.

2.3 Other hazards - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.2 Mixtures

Component		Classification	Concentration
Platinum****			
CAS-No.	7440-06-4	Flam. Sol. 1; H228	< 10 %

Riogen
Catalysis for Chemicals & Energy

Safety Data Sheet (SDS)

Issue date: 03/04/2015

1. Product and Company Identification

Product name: 20% Nickel on γ-Alumina (20 wt% Ni/Al_2O_3)
Product codes: 0040-ALNiA20-Powder-25G
Intended use: Multiple uses in Research and development, Laboratory chemicals, Manufacture of substances
Supplier: 107 Gilbreth Parkway, Suite 103, South Jersey Technology Park, Mullica Hill, NJ 08062
Email: info@riogeninc.com
Emergency Telephone: 609-606-2012

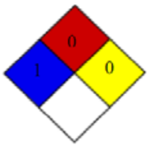
2. Composition, Information on Ingredients

Chemical characterization: Mixture (20 wt% Nickel on 80 wt% Aluminium oxide, reduced)
Chemical components:
- **Alumina or aluminium oxide: CAS#** 1344-28-1
- **Nickel: CAS#** 7440-02-0

Non Hazardous Ingredients: None
Risk Phrases: None listed
Additional Information: None known

3. Hazards Identification

Classification of the substance or mixture: This material is a non flammable solid and limited evidence of carcinogenic effect, harmful if inhaled, may cause an allergic skin reaction and respiratory irritation.
Signal word: Not applicable
Emergency overview: This product is a dark/grey solid. In case of fire: Use carbon dioxide, dry sand, dry chemical or alcohol-resistant foam for extinction. Wear protective gloves, protective clothing, eye/face protection.
Potential health effects: May cause irritation in the event of inhalation, eye contact, skin contact or ingestion. Limited evidence of carcinogenic effect.
HMIS (Hazardous Materials Identification System) ratings (Scale 0-4)



Health	1
Fire	0
Reactivity	0

Other Hazards: Not applicable

4. First Aid Measures

Description of First aid measures: Consult doctor immediately and show this safety data sheet.
Inhalation: If inhaled in, supply fresh air. If not breathing, give artificial respiration. Consult a physician.
Eye contact: Rinse eye with running water for several minutes then consult doctor immediately.
Skin contact: Wash with water and soap and rinse thoroughly for several minutes
Ingestion: Rinse mouth with water and seek medical treatment immediately.
Notes to Physician: No information available