

Exploring beneficial uses of *Ecklonia maxima* waste residue

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PREFACE AND ACKNOWLEDGMENTS

Organic waste offers a high incentive when turned into a resource. The high output of organic waste in South Africa calls for innovative sustainable ways of diverting it from disposal to landfill and moving it up the value chain thus greening the economy. The research was focused on finding the most applicable beneficial applications/uses of organic seaweed waste residue. Seaweed waste is produced by a manufacturing company in South Africa at a rate of 15 – 20 tonnes per day. The ideal circular economy trait would be to return the waste back into the “circular economy”, thereby moving away from landfilling towards the optimum utilisation of resources.

My utmost gratitude goes to my supervisor, Dr Claudine Roos for her invaluable patience and guidance throughout my Masters journey. I also extend my gratitude to the North-West University, Unit for Environmental Sciences and Management for their unwavering support. My studies would not have been possible without the generous support from the North-West University bursary department.

I am also thankful to my classmates and work colleagues for their encouragement and support.

Unto Him who is able to do exceedingly and abundantly, above all that I could think or imagine. In Him, I live, I move and I have my entire being.

ABSTRACT

The rise in technological advancements has brought about an increase in industrial production, this in turn has caused a surge in consumerism, with waste being generated at an increasing rate in a linear economy system. The increase in waste generation has resulted in pressures on landfill sites and economic resources being directed to the management of these landfills. To address the adverse impacts associated with the disposal of waste and to divert waste away from landfilling, the South African government has developed legislation, strategies and regulations towards implementing the waste management hierarchy, with specific focus on the beneficial use of waste and achieving a circular economy.

The aim of this research was to explore the potential beneficial uses of *Ecklonia maxima* waste residue generated by a seaweed processing company based in South Africa. This was achieved through a mixed methods approach. In summary, the physico-chemical properties of *Ecklonia maxima* waste residue were determined; the possible beneficial uses of *Ecklonia maxima* waste residue were identified and ranked to identify the most preferable use of the waste residue; and the optimal conditions of the most preferred identified beneficial use, were ultimately, evaluated.

Laboratory analysis of the waste residue, supported by literature review, indicated the presence of nutritional elements in the algae waste residue (nitrogen: 2.18%, potassium: 5.58%, phosphorus: 0.35%, and organic matter: 63.26% (based on dry mass)). These characteristics of the waste residue make it potentially suitable for beneficial use in the production of animal feeds and aquaculture; the food sector; biogas production; use as a soil conditioner or compost additive; as well as potential reprocessing of the waste residue in production processes of the seaweed processing company (after enzymatic hydrolysis).

Based on a ranking process (which applied criteria such as suitability of algae waste residue composition for beneficial use, compliance of the beneficial use within the South African legal framework, economic viability, energy efficiency, business advantage, and compatibility of the beneficial use with existing formulations) enzymatic hydrolysis for reprocessing was identified as the most preferred beneficial use for the algae waste residue.

The research, finally, evaluated the optimal conditions for the enzymatic hydrolysis of algae waste residue for the purposes of reprocessing. Different enzyme- and pre-treatment combinations were tested to determine the combination which would yield the highest concentration of total soluble organic matter and total sugars. Residues pre-treated with peracetic acid, generally yielded higher concentrations of total organic matter and total sugars, than residues treated with sulphuric acid, or those without any pre-treatment. The enzyme combination of Celluclast + Viscozyme yielded the highest concentration of total organic matter and sugars.

An in-depth analysis of the *E. maxima* waste residue is critical in exploring all the potential beneficial uses. Future laboratory analysis should include the full profile of all the complex carbohydrates in the residue. More research should be conducted in assessing the suitability of the waste residue in relation to its inclusion in the food manufacturing sector. Finally, process optimisation of enzymatic hydrolysis for reprocessing purposes is recommended.

Keywords: *alternatives, beneficial use, Ecklonia maxima, waste residue, seaweed waste, circular economy*

ABBREVIATIONS AND ACRONYMS

AAFCO	Association of American Feed Control
AFL	Agriculture and Food Laboratory
AI	Allowable intake
AOAC	Association of Official Analytical Chemists
C-C links	Carbon to Carbon links
CIRS	Centre for Innovation in Regulatory Science
CLP	Classification, Labelling and Packaging
C:N	Carbon nitrogen ratio
DEA	Department of Environmental Affairs
DEA & DP	Department of Environmental Affairs & Department of Planning
DEFF	Department of Environment, Forestry and Fisheries
DFFE	Department of Forestry, Fisheries and Environment
DUMAS	Jean-Baptiste Dumas (testing method named after him)
EC	Electrical conductivity
EC	European Commission
ECHA	European Chemicals Agency
EPA	United States Environmental Protection Agency
EPR	Extended Producer Responsibility
EU	European Union
FAO	Food and Agricultural Organisation Agency
FNB	Food and Nutrition Board
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GN R	Government Notice Regulation
HM	Heavy metals
ICP	Inductively Coupled Plasma
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry

ISO	International Standards Organisation
MPL	Maximum permissible level
MSDS	Material Safety Data Sheet
MTL	Maximum tolerable limit
NEM: WA	National Environmental Management: Waste Act
NWMS	National Waste Management Strategy
NPK	Nitrogen Phosphorus Potassium
NRC	National Research Council
PAA	Peracetic Acid
UN	United Nations,
RDA	Recommended daily allowance
RO	Research Objective
RQ	Research Question
RSA	Republic of South Africa
SA	South Africa
SANAS	South African National Accreditation System
SANS	South African National Standard
SAWIC	South African Waste Information Centre
SAWIS	South African Waste Information System
SDS	Safety Data Sheet
SGS	Société Générale de Surveillance Laboratory
TOC	Total Organic Carbon
TOM	Total Organic Matter
UN	United Nations
UNEP	United Nations Environment Programme
USD	United States Dollars
USEPA	United States Environmental Protection Agency
WHO	World Health Organisation

List of acronyms/abbreviations for elements

As	Arsenic
B	Boron
C	Carbon
Ca	Calcium
Cd	Cadmium
Cl	Chlorine
Cl ⁻	Chloride
Cu	Copper
Co	Cobalt
Cr	Chromium
Fe	Iron
HCl	Hydrochloric acid
H ₂ SO ₄	Sulphuric acid
Hg	Mercury
I	Iodine
K	Potassium
Mg	Magnesium
Mo	Molybdenum
N	Nitrogen
Na	Sodium
Ni	Nickel
P	Phosphorus
Pb	Lead
S	Sulphur
Se	Selenium

Units of measurement

°C	degrees Celsius
dS/m	decisiemens per metre
g	grams
g/L	grams per litre
g/kg	grams per kilogram
kg	kilograms
mcg	micrograms
mg/L	milligrams per litre
mg/kg	milligrams per kilogram
mM	milli molar
µg/g	micrograms per gram
ppb	parts per billion

KEY DEFINITIONS

Anaerobic digestion

The breaking down of organic matter by microorganisms in the absence of oxygen.

Ross et al., 2007

Beneficial use

Valuable application of a product or material

Golev et al., 2016

Biogas

Refers to the mixture of gases produced by the breakdown of organic matter in the absence of oxygen (anaerobically), primarily consisting of methane and carbon dioxide.

National Waste Management Strategy (2020)

Compost

A product of controlled aerobic, biological decomposition of biodegradable materials. The organic waste undergoes mesophilic and thermophilic temperatures, which significantly reduces the viability of pathogens and weed seeds, and stabilizes the carbon such that it is beneficial to plant growth.

GN R 1132 of 2019

Composting

Means a controlled biological process in which organic materials are broken down by micro-organisms by means of an aerobic process to produce compost or fertiliser.

GN R 1132 of 2019

Circular economy

An economy that is restorative and regenerative by design and aims to keep products, components, and materials at their highest utility and value at all times, distinguishing between technical and biological cycles.

National Waste Management Strategy (2020)

Ecklonia Maxima

Ecklonia maxima is a species of brown algae in the family Lessoniaceae

Lotze & Hoffman, 2016

Enzymatic hydrolysis

It is a technology that makes use of enzymes to breakdown complex compounds into smaller soluble chains ie Cellulase breaking down cellulose into sugar chains.

Charoensiddhi et al., 2016

Extended producer responsibility

Refers to an environmental policy approach in which a producer's responsibility for a product is extended to the post- consumer stage of a product's life

National Waste Management Strategy (2020)

Fertilizer

Means any substance which is intended or offered to be used for improving or maintaining the growth of plants or the productivity of the soil.

GN R 732 of 2012

Recycle

Means a process where waste is reclaimed for further use, which process involves the separation of waste from a waste stream for further use and the processing of that separated material as a product or raw material.

NEM: WA (59 of 2008)

SANS 10234

The latest edition of the South African National Standard Globally Harmonized System of Classification and labelling of Chemicals (GHS)

GN R 634 of 2013

Waste

(a) any substance, material or object, that is unwanted, rejected, abandoned, discarded or disposed of, or that is intended or required to be discarded or disposed of, by the holder of that substance, material or object, whether or not such substance, material or object can be re-used, recycled or recovered and includes all wastes as defined in Schedule 3 to this Act; or (b) any other substance, material or object that is not included in Schedule 3 that may be defined as a waste by the Minister by notice in the Gazette, but any waste or portion of waste, referred to in paragraphs (a) and (b), ceases to be a waste—(i) once an application for its re-use, recycling or recovery has been approved or, after such approval, once it is, or has been re-used, recycled or recovered; (ii) where approval is not required, once a waste is, or has been re-used, recycled or recovered; (iii) where the Minister has, in terms of section 74, exempted any waste or a portion of waste generated by a particular process from the definition of waste; or (iv) where the Minister

has, in the prescribed manner, excluded any waste stream or a portion of a waste stream from the definition of waste.

NEM: WA (59 of 2008) as amended

Any substance, material or object-

That the generator of that substance, material or object has no further use for within its own processes, whether or not it has any commercial value for the generator, but which can be re-used, recycled, recovered or traded in by any person; or

That is rejected, abandoned, discarded or disposed of, either temporary or permanently, or is intended to be discarded or disposed of by the generator of that substance, material or object, regardless of whether or not that substance, material or object has any commercial value for the generator or can be re-used, recycled, recovered or traded in by any person; or

(b) Any other substance, material or object that may be defined as a waste by the Minister by notice in the Gazette; but any waste or portion of waste, referred to in paragraph (a) and (b) ceases to be a waste-

(aa) once it is re-used, recycled or recovered or traded in by the holder of the waste or portion of waste in accordance with a condition stipulated in a valid waste management licence, where applicable, or in accordance with an applicable norm or standard made in terms of this Act; or

(bb) where the Minister has, in the prescribed manner, excluded the holder of any waste stream or a portion of a waste stream from the definition of waste, enabling the holder thereof to trade in the excluded waste stream or portion of the excluded waste stream, provided that the holder has satisfied the requirements of proving the environmentally safe use of the waste stream or portion of the waste stream by it or any other person and committed to provide the Minister with annual reports of the use thereof.

NEMLA, 2022 (Act 2 of 2022)

Waste beneficiation

Refers the treatment of waste to improve its physical or chemical properties to use it as a raw material into production processes and extracting economic value.

National Waste Management Strategy (2020)

Waste classification

establishing (a) whether a waste is hazardous based on the nature of its physical, health and environmental hazardous properties (hazard classes); and (b) the degree of severity of hazard posed (hazard categories).

GN R 634 of 2013

TABLE OF CONTENTS

PREFACE AND ACKNOWLEDGMENTS	I
ABSTRACT	II
ABBREVIATIONS AND ACRONYMS	III
KEY DEFINITIONS	VII
CHAPTER 1 INTRODUCTION	1
1.1 Background	1
1.1.1 Seaweed processing and resultant waste streams	1
1.1.2 Major components of the seaweed industry	1
1.1.3 Seaweed processing	2
1.1.4 Wastes generated during seaweed processing.....	3
1.1.5 Waste management and the strive towards beneficial use of waste	4
1.2 Problem statement and rationale for the study	5
1.3 Research aim and objectives.....	6
1.4 Scope of the research	7
1.5 Assumptions and limitations.....	7
1.6 Potential contribution of the research	8
1.7 Structure and outline of the dissertation	8
1.8 Conclusion.....	9
CHAPTER 2 LITERATURE REVIEW.....	10
2.1 Introduction	10
2.2 Application of seaweed in the agricultural sector.....	10

2.2.1	<i>Ecklonia maxima</i> (Giant Kelp).....	11
2.3	Properties and composition of <i>E. maxima</i> waste residue	12
2.3.1	Physical characteristics and organic matter (Proximate analysis)	12
2.3.2	Elemental analysis.....	12
2.3.3	Heavy metal content.....	13
2.3.4	Biologically active compounds	13
2.3.4.1	Polysaccharides	13
2.3.4.2	Proteins	14
2.3.4.3	Phlorotannins	15
2.3.4.4	Fucoxanthin.....	15
2.3.4.5	Polyols.....	15
2.3.4.6	Betaines	15
2.4	South Africa legal framework related to beneficial use of waste	16
2.4.1	National Environmental Management Waste Act (NEM:WA) (59 of 2008)	17
Figure 2-2: The waste management hierarchy, as proposed by the National Waste Management Strategy (Adapted from DEFF, 2020)		
		18
2.4.2	The National Waste Management Strategy (NWMS).....	18
2.4.3	Circular economy.....	19
2.4.4	Waste Classification and Management Regulations	19
2.4.5	South Africa landfill restrictions on seaweed waste residue (biomass).....	21
2.4.6	Regulations regarding the exclusion of a waste stream or a portion of a waste stream from the definition of waste (GN 715 of July 2018).....	21
2.4.7	Extended Producer Responsibility (EPR) Regulations (GN. 400 of May 2021)	22

2.4.8	National norms and standards composting of organic waste (GN. 591 of June 2021)	22
2.4.9	Western Cape Government: Organic waste disposal to landfill restrictions	22
2.5	International waste to landfill restrictions	22
2.5.1	European restrictions.....	23
2.5.2	Zimbabwean restrictions.....	23
2.5.3	Moroccan restrictions.....	23
2.6	Alternatives uses of <i>Ecklonia maxima</i> waste residue.....	24
2.6.1	Aquaculture and animal feeds	24
2.6.2	Food sector: Source of flavourant and nutrition.....	25
2.6.3	Biogas production.....	26
2.6.4	Soil Conditioner/ Compost	26
2.6.5	Enzymatic hydrolysis of seaweed residue for reprocessing of waste residue....	27
2.7	Factors for determining the acceptability and feasibility of beneficial uses of seaweed residue waste.....	27
2.8	Chapter summary	29
CHAPTER 3 METHODOLOGY		30
3.1	Introduction	30
3.2	Research design.....	30
3.3	Data collection	32
3.3.1	Laboratory analysis to determine the composition of <i>E. Maxima</i> waste residue	32
3.3.2	Literature review	33

3.3.2.1	Literature review to supplement composition information on <i>E. maxima</i> waste residue	34
3.3.2.2	Literature review to identify the possible beneficial uses.....	34
3.3.2.3	Literature review to determine the criteria which should be taken into account when ranking beneficial uses for their preferability	35
3.3.3	Ranking of beneficial uses	37
3.3.4	Determining the optimal conditions for the most preferred identified beneficial use of <i>E. maxima</i> waste residue: Enzymatic hydrolysis of <i>E. maxima</i> waste residue	39
3.3.4.1	Method description	40
3.3.4.1.1	Controls	40
3.3.4.1.2	Pre-treatment.....	40
3.3.4.1.3	Enzymatic hydrolysis	40
3.3.4.1.4	pH variations	41
3.4	Data analysis.....	41
3.4.1	<i>E. maxima</i> waste residue composition (Literature review and SGS laboratory)	41
3.4.2	Alternative beneficial uses of <i>E. maxima</i> waste residue (Literature review)	41
3.4.3	Results from enzymatic hydrolysis of <i>E. maxima</i> waste residue laboratory trial	42
3.5	Ethical considerations	42
3.6	Methodological assumptions and limitations.....	42
3.7	Chapter summary	43
CHAPTER 4	RESULTS AND DISCUSSION	44
4.1	Introduction	44

4.2	Physico-chemical composition and characteristics of <i>Ecklonia maxima</i> waste residue and its implications for beneficial use.....	45
4.2.1	Complex compound composition (from literature review).....	46
4.2.1.1	Polysaccharide composition of <i>E. maxima</i> waste residue and implications for beneficial use	46
4.2.1.2	Protein content of <i>E. maxima</i> waste residue and implications for beneficial use	47
4.2.2	Laboratory analysis of <i>E. maxima</i> waste residue and its implications for beneficial use	48
4.2.2.1	Physical characteristics	48
4.2.2.1.1	Implications of physical characteristics for use in animal feeds and aquaculture.....	49
4.2.2.1.1.1	Implications of physical characteristics for use in the food sector (flavourant and nutrient)	50
4.2.2.1.1.2	Implications of physical characteristics for biogas production	50
4.2.2.1.1.3	Implications of physical characteristics for use as soil conditioner/compost additive	51
4.2.2.1.1.4	Implications of physical characteristics for enzymatic hydrolysis and re-processing for further compound extraction	51
4.2.2.2	Elemental analysis (excluding heavy metals) of <i>E. maxima</i> waste residue	51
4.2.2.2.1	Implications of elemental composition for animal feeds and aquaculture	52
4.2.2.2.2	Implications of mineral composition for use in the food sector (flavourant and nutrient)	53
4.2.2.2.3	Implications of mineral composition for biogas production	53
4.2.2.2.4	Implications of mineral composition for use as soil conditioner/compost additive	54

4.2.2.2.5	Implications of mineral composition for enzymatic hydrolysis and re-processing for further compound extraction	54
4.2.2.3	Heavy metal and iodine content.....	54
4.2.2.3.1	Implications of heavy metal and iodine composition for aquaculture and animal feeds	55
4.2.2.3.2	Implications of heavy metal and iodine composition for use in the food sector (flavourant and nutrient)	56
4.2.2.3.3	Implications of heavy metal and iodine composition for biogas production.....	58
4.2.2.3.4	Implications of heavy metal and iodine composition for use as soil conditioner/compost additive	58
4.2.2.3.5	Implications of heavy metal composition for enzymatic hydrolysis and re-processing for further compound extraction	60
4.3	Ranking possible beneficial uses to determine the most preferred beneficial use (RO2)	60
4.3.1	Criteria 1: Suitability of composition for beneficial use	61
4.3.2	Criteria 2: Compliance with South African legal framework	62
4.3.3	Criteria 3: Moving waste up the waste management hierarchy and Criteria 4: enabling a circular economy	64
4.3.4	Criteria 5: Economic viability.....	65
4.3.5	Criteria 6: Energy efficiency	66
4.3.6	Criteria 7: Business advantage and compatibility with the strategic direction of the organisation	66
4.3.7	Criteria 8: Compatibility with existing formulations	67
4.3.8	Criteria 9: Practical feasibility.....	67
4.3.9	Summary: Enzymatic hydrolysis for reprocessing of waste residue as the most preferred beneficial use.....	68

4.4	Determining the optimal conditions for enzymatic hydrolysis for reprocessing of <i>Ecklonia maxima</i> waste residue (RO3)	70
4.4.1	Pre-treatment results	70
4.4.2	Enzymatic hydrolysis	73
4.4.2.1	Total organic matter (TOM) yield	73
4.4.2.2	Sugar yield	74
4.5	Chapter summary	76
 CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS		78
5.1	Introduction	78
5.2	Conclusions	78
5.2.1	Conclusions related to RO 1: Determining the physico-chemical composition and characteristics of <i>Ecklonia maxima</i> waste residue.....	78
5.2.2	Conclusions related to RO 2: Identifying the possible beneficial uses of <i>Ecklonia maxima</i> waste residue (based on the findings of RO1), and ranking these beneficial uses to determine the most preferred beneficial use	79
5.2.3	Conclusions related to RO 3: Determining the optimal conditions of the most preferred identified beneficial use of <i>Ecklonia maxima</i> waste residue	80
5.3	Recommendations and areas of future research	80
 BIBLIOGRAPHY		82
 APPENDIX 1		97

LIST OF TABLES

Table 2-1: Legislation applicable to the management of algae waste residue, and its potential beneficial use, within the South African context.	16
Table 3-1: Research design and data collection methods	31
Table 3-2: Analytical methods used to determine the composition of <i>E. maxima</i> waste residue.....	33
Table 3-3: Ranking table/matrix used to rank the different beneficial use options for <i>E. maxima</i> waste residue, to ultimately determine the most preferred beneficial use option.	37
Table 3-4: Enzymatic hydrolysis enzyme and pre-treatment combinations (Charoensiddhi <i>et al.</i> , 2014)	39
Table 4-1: Physical characteristics and organic content of <i>E. maxima</i> waste residue (SGS Somerset West 22C C022, 2022)	49
Table 4-2: Elemental analysis (excluding heavy metals) of <i>E. maxima</i> waste residue (SGS Somerset West 22C C022, 2022)	52
Table 4-3: Beneficial minerals/elements with a maximum tolerable level (MTL) applicable to cattle by NRC (NRC, 2005)	52
Table 4-4: Comparative table RDA/AI (in accordance to WHO/FAO) for adults vs mineral composition in <i>E. maxima</i> waste residue (Koubova <i>et al.</i> , 2018; National Institute of Health, 2022)	53
Table 4-5: Heavy metals (non-beneficial elements) and iodine of <i>E. maxima</i> waste residue (SGS AFL MT20-01010: 2020; SGS Somerset West 22C C022, 2022).....	55
Table 4-6: Maximum Tolerable Level (MTL) of heavy metals according to the US National Research Council (NRC) & Association of American Feed Control (AAFCO) and heavy metals (none beneficial) in <i>E. maxima</i> waste residue (Dai <i>et al.</i> , 2016; Deemy & Benjamin, 2019).....	56

Table 4-7:	Maximum permissible level (MPL) of heavy metal in fish feed vs heavy metals in waste residue (Mannzhi <i>et al.</i> , 2021).....	56
Table 4-8:	Recommended dietary allowances (RDA) for iodine (National Institute of Health (US), 2022)	57
Table 4-9:	Heavy metal limits allowed in organic fertilisers in Brazil, USA and EU (Klinglmair & Thomsen, 2020; Souza <i>et al.</i> , 2018)	59
Table 4-10:	World Health Organisation (WHO) heavy metal limits allowed soil (Zondo, 2021)	59
Table 4-11:	Criteria for acceptance of <i>E. maxima</i> waste residue beneficial use	61
Table 4-12:	Acceptable levels of heavy metals (HM) and iodine allowed in food, soil and ruminant feeds (Department of Health, 2006:114; Nkosi <i>et al.</i> , 2021; Zondo, 2021).....	63
Table 4-13:	Sugar profile yield comparison showing impact of pre-treatment on yield.....	74

LIST OF FIGURES

Figure 1-1: Manufacturing flow diagram from seaweed (Company archives) 3

Figure 4-1: Pre-treatment supernatant total organic matter (TOM) yield results 71

Figure 4-2: Pre-treatment supernatant sugar yield results 72

Figure 4-3: Enzymatic yield (TOM) comparison, pre-treatment with PAA vs 0.1%
H₂SO₄ 73

Figure 4-4: Enzymatic yield (Total sugar yield) comparison, pre-treatment with 26%
PAA vs 0.1% sulphuric acid 74

CHAPTER 1 INTRODUCTION

1.1 Background

This section provides the background to this research, with focuses on *Exploring the beneficial uses for Ecklonia maxima (seaweed) waste residue*. The first sub-section provides background on the processing of seaweed and its resultant waste streams, while the second sub-section provides a brief overview of the global waste management situation and the strive towards considering the beneficial use of waste.

1.1.1 Seaweed processing and resultant waste streams

The extensive use of macro and micro algae has risen over the years, with significant research on the use of algae (which includes seaweed) having started around the World War II era (Craigie, 2011). Research undertaken in 2009 by Chopin and Sawhney indicated that the seaweed industry was at a value slightly under six billion US dollars globally (Craigie, 2011).

1.1.2 Major components of the seaweed industry

The seaweed industry can be broken down into four major components:

- **Phycocolloids (seaweed gum):** The main phycocolloids found in seaweeds are agar, alginates and carrageenan. These compounds are well incorporated within the manufacturing sector because of their colloidal properties (Lomartire *et al.*, 2021). The aforementioned compounds play a significant role in the food production sector as well as in technological advancement applications (Craigie, 2011).
- **Sea vegetables:** Certain seaweed varieties, for instance, *Undaria pinnatifida* have been consumed by humans. This practice of eating seaweeds was first popular within the Asian communities, only recently spreading into the Western communities (Lomartire *et al.*, 2021; Dhargalkar & Pereira, 2005). Seaweeds are rich in dietary fibre and certain phytocompounds hence the rise in seaweed consumption (Dhargalkar & Pereira, 2005).
- **Phycosupplements:** Extraction of essential nutrients and elements from seaweed is a practice that is becoming more prevalent as the awareness of the importance of seaweed spreads. Iodine, vitamins, and folic acid seaweed supplement capsules are typical examples of the use of seaweeds in the phycosupplement field (Dhargalkar & Pereira, 2005).

- **Plant bio stimulants:** Seaweeds have been found to contain plant hormones such as Auxins, Gibberellins and Cytokinin, these growth hormones are essential for plant growth and development (Nabti *et al.*, 2009).

In 2010, Bixler and Porse placed the seaweed-derived polysaccharides (such as, alginates, cellulose and dietary fibre) industry at a value of one billion US dollars, with agriculture-related seaweed products being worth about fifty million US dollars (Craigie, 2011). To date the market is valued at USD 15.01 billion, it is expected to have grown to USD 24.92 billion by 2028 (Fortune business insights, 2021).

1.1.3 Seaweed processing

There are various ways of producing seaweed extracts that can be followed. The method of manufacture determines the final product, as well as the waste that is eventually generated. Some common methods of seaweed processing include:

- Acid hydrolysis (uses hydrochloric acid, sulphuric acid etc);
- Cell burst techniques (pressure differentials to rupture the cell walls); and
- Alkaline hydrolysis (using sodium hydroxide, or any other suitable base) (Lotze & Hoffman, 2016).

The production process for a seaweed processing company, using the cellburst method, is outlined in Figure 1-1. The process includes:

1. The harvesting site is along the coastline of the Cape-Point National Park which is a conservation area. Divers sustainably hand-harvest the fresh seaweed "*Ecklonia maxima*" on a strip rotational basis from the cold, clean waters of the Atlantic Ocean. The harvesting program ensures a uniform age and size of the raw material used. Once harvested, seaweed is immediately transported in temperature-controlled trucks to the processing site where it is immediately sorted according to stipes (stems) and fronds (leaves).
2. The stipes are chopped with knives and reduced to smaller fractions.
3. The stipes and fronds are washed separately with clean water.
4. The stipes and fronds are mixed at a specific ratio and reduced to a mince consistency.
5. Minced seaweed is further reduced to a desired finer particle size and a small amount of preservative is added (natural vinegar).
6. The micronised seaweed goes through the cold cellular burst process where the cell walls are ruptured to release the contents by means of pressure differentials. Cell burst seaweed is mixed with sterile water and additional preservative (natural vinegar).

- The mixture is then centrifuged at a high speed to separate the liquid seaweed extract from the solids (seaweed residue). The seaweed residue then becomes the organic waste, an average of 20 tonnes of spent seaweed is produced per day.

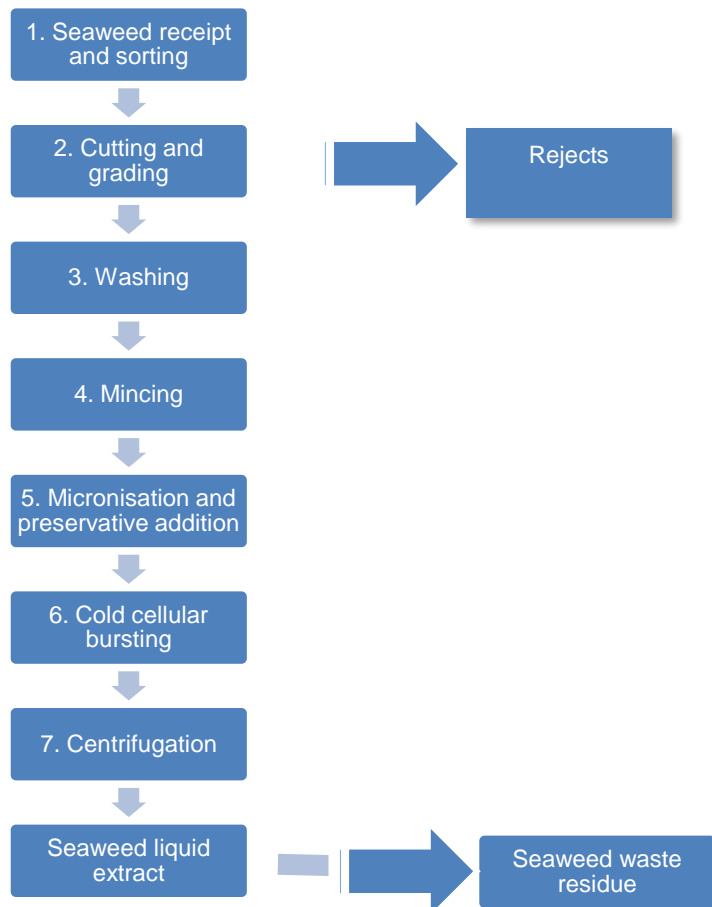


Figure 1-1: Manufacturing flow diagram from seaweed (Company archives)

1.1.4 Wastes generated during seaweed processing

The extraction process during seaweed processing determines the biological molecules that remain active in the final product. Factors like target crops, season and crop age play a pivotal role in plant bio stimulant manufacturing process (Boukhari, 2020).

In China, the extraction of alginates from Kelp (*Laminaria japonica Aresch*) leaves behind a crude waste that is rich in fibres, protein and a small amount of alginates. This waste residue has the potential of being degraded to produce organic nutrients and mineral nutrients for plant growth and development. At present, this waste is currently being landfilled thus wasting natural resources and much need land space (Zheng *et al.*, 2016).

The use of mineral acids during the manufacturing process results in the complete or partial solubilisation of the fibre, this results in a liquid final product with little to no waste residue, however this process is not safe for the environment as mineral acids such as Sulphuric acid and Hydrochloric acid are harsh. These acids result in an acidic seaweed extract that will require pH correction (Godlewska *et al.*, 2016).

The type of waste produced in seaweed processing industries is heavily dependent on the method of manufacture. Majority of seaweed processing facilities either use mechanical methods of manufacture or chemical methods of manufacture. Companies in the organic manufacturing sector seldom use harsh chemicals like mineral acids or bases (organic acids being the exception to the rule, these include acetic acid, lactic acid, uric acid etc), this is because most of the bioactive compounds within seaweed are very sensitive to harsh chemicals and processes (Laohakunjit *et al.*, 2014).

In the case of the seaweed processing company under investigation, the research was focused on *Ecklonia maxima* waste residue.

1.1.5 Waste management and the strive towards beneficial use of waste

Globally, the majority of the production systems are still quite linear. Resources are extracted and processed into goods that are discarded immediately after use. This “linear economy” is characterised by large quantities of waste being generated and coupled with resource depletion. (Vaibhav *et al.*, 2020). The strip rotational harvesting of seaweed is not always adhered to, in such cases resource depletion then occurs (Taelman., *et al* 2015)

Internationally, landfill disposal (i.e. “linear economy”) is still the norm. Landfilling, if not properly managed and implemented, can result in adverse impacts on the environment and negative impacts on human health. Landfill gas emissions are also a significant contributing factor to global warming and climate change (Cudjoe & Han 2020). However, many countries are implementing measures to reduce the amount of waste that is being disposed in landfills. European countries have been making significant progress in reducing the amount of solid waste that is disposed to landfill. This is particularly true for countries like Sweden, Finland, Netherlands and France, to name a few (Vaverrkova, 2019).

In 2017, South Africa generated a total of about 55.6 million tonnes of general waste, of which approximately 65.2% was disposed to landfill. Of the 65.2% that was landfilled most of it was organic waste that could have been reintroduced into the production system (DEA, 2018). Little regard being given to the waste management hierarchy (UNEP, 2018).

In 2008, the European Union (EU) under Directive 2008/98/EC (Waste Framework Directive) introduced the waste management hierarchy. This move later on led to the waste management hierarchy being incorporated in the laws of the EU member states (Martinho & Pires 2019). Within the EU, the waste management hierarchy is further emphasised in their Circular Economy Action Plan, hence the significant shift in reduction of waste to landfill that is being witnessed within the EU member states (Martinho & Pires 2019). In 2016, the United Nations included the waste management hierarchy in their Sustainable Development Goals under Goal 12 (UN, 2016).

The 2020 South African National Waste Management Strategy highlights the importance to move from a “linear” to a “circular economy” through the implementation of the waste management hierarchy (DEFF, 2020).

1.2 Problem statement and rationale for the study

The increased generation and associated landfilling of waste has become an issue of global concern (UNEP, 2016). Population growth, rapid urbanisation and a steady growth of the manufacturing industry have caused a surge in the waste generated per capita in South Africa (Muzenda, 2014; SAWIC, 2021). This causes pressure on waste management infrastructure and landfill sites (Vijayakumar & Sebastian, 2018). If not properly regulated, landfill disposal may cause significant adverse environmental-, human health-, social- and economic impacts. The depleting natural resources and the rising cost of living requires for the responsible personnel to come up with innovative ways to encourage sustainable manufacturing practices. A linear economy has caused a surge in the depletion of resources as well as a huge accumulation of waste on land.

In the South African context, the National Environmental Management Waste Act (Act 59 of 2008) (NEM:WA) aims to avoid and manage the negative impacts of waste. Since the promulgation of the NEM:WA in 2009, the government of South Africa has published a plethora of regulations and policies to assist in achieving the aims of the Act. The National Waste Management Strategy (NWMS) (2020) is a policy document which aims to achieve the objectives of the NEM:WA. The 2020 NWMS has three key pillars, with Pillar 1 focusing on waste minimisation and the diversion of waste from landfilling. In essence, Pillar 1 of the NWMS focuses on the implementation of the waste management hierarchy, where the re-use, recycling and recovery of waste are favourable options, with landfilling being the least favourable option (DEFF, 2020). The NWMS further promotes the circular economy concept, where waste is regarded as a resource, which could be of beneficial use. In line with the principles of the waste management hierarchy, and to promote a circular economy, the *Regulations regarding the exclusion of a waste stream or a portion of a waste stream from the definition of waste (GN. 715 of 18 July 2018)* were promulgated in 2018.

These regulations offer a means to eliminate a type of waste from the description of waste. Furthermore, the requirements applicable to 'extended producer responsibility' have also been formally regulated in terms of the *Regulations regarding extended producer responsibility (GN. 1184 of November 2020)*. These regulations necessitate Extended Producer Responsibility (EPR) schemes for the re-use, recycling and recovery for valuable use of the products identified by the Minister.

In the context of the above, also considering the depletion of natural resources and the rising costs of waste management, industry needs to find innovative ways to encourage sustainable manufacturing- and waste management practices, which considers beneficial use, and incorporates the principles of a 'circular economy' and 'extended producer responsibility'.

This research specifically aims to explore beneficial uses of *Ecklonia maxima* residue – a waste stream generated by a seaweed processing company located in Cape Town, South Africa. Due to propriety information, the seaweed processing company will remain anonymous. The company specialises in the production of plant bio-stimulants for the local and international market. *Ecklonia maxima* is the company's primary ingredient, with various formulations based on this seaweed variety. It is worth noting that the company in question is Ecocert accredited (organic farming accrediting body) thus it produces organically certified formulations.

The company produces an average of twenty (20) tonnes of seaweed waste residue per day. The residue has no foreign additives, thus, it is classified as a non-hazardous organic residue. At present the waste stream is either donated to specific companies for further use, or given to a third party to discard the waste. Seaweed waste residue is an organic waste stream, which has the potential to be moved up the waste management hierarchy (away from disposal) (Alfosin *et al.*, 2018).

Some stakeholders (European clients) require the company to show their support for the Circular Economy Action Plan that was published in March 2020 (European Commission, 2020). The company must provide proof of the various projects that it is undertaking to reduce waste and to manufacture sustainably. Exploring alternatives to landfilling of *Ecklonia maxima* waste residue may have the benefits of decreasing disposal impacts, whilst also decreasing reliance on raw/virgin materials (Nyika *et al.*, 2014), to ultimately promote the waste management hierarchy and achieve circular economy principles.

1.3 Research aim and objectives

The aim of this research is to explore the beneficial uses of *Ecklonia maxima* waste residue, which is generated by a seaweed processing facility based in South Africa.

In line with the aim, the following objectives are set:

- **Research objective 1:** Determining the physico- chemical composition and characteristics of *Ecklonia maxima* waste residue;
- **Research objective 2:** Identifying the possible beneficial uses of *Ecklonia maxima* waste residue (based on the findings of RO1), and ranking these beneficial uses to determine the most preferred beneficial use;
- **Research objective 3:** Determining the optimal conditions of the most preferred identified beneficial use (identified in RO2) of *Ecklonia maxima* waste residue.

1.4 Scope of the research

The research focuses on finding possible beneficial uses of *Ecklonia maxima* waste residue produced by a seaweed processing company based in Cape Town, South Africa. Potential beneficial uses are based on (1) the physico-chemical composition of the waste stream, as well as (2) known beneficial/alternative uses of similar waste streams, in South Africa and internationally.

The research was conducted between February and October of 2022.

In the context of this research, *beneficial use* refers to the use of *Ecklonia maxima* waste residue, by the seaweed processing company (internally), as an alternative to landfill disposal or donation of the waste to external companies – with a view of, ultimately, achieving the principle of a “circular economy”.

The beneficial use options for *Ecklonia maxima* waste residue are ranked from most to least preferred, based on desirability and feasibility criteria, which consider factors such as the suitability of the waste for the beneficial use, compliance to the South African legal framework, cost and financial viability, business advantage, compatibility with current formulations, etc.

Only the most preferred beneficial use is tested further by an accredited laboratory, to determine the optional conditions for the beneficial use of *Ecklonia maxima* waste residue.

1.5 Assumptions and limitations

Majority of the research that has been done on alternatives/beneficial use of wastes have been conducted outside the South African context (Muedi, 2021) and will have to be feasible and desirable within the country, given our unique conditions, compared to the developed world.

The research is limited to literature review, document analysis and laboratory analysis. Feasibility and desirability were determined based on company policy. No broad-based stakeholder interviews were included in the scope of this research.

Only the most preferred (based on company-specific desirability and feasibility criteria) was evaluated further, to determine the optimal conditions for the beneficial use option, due to financial and time constraints.

1.6 Potential contribution of the research

Insufficient knowledge exists on the potential beneficial uses of waste, (Noorjahan *et al.*, 2020), especially *Ecklonia maxima* waste residue. *E. maxima* is predominantly located in the south shallow waters of the western side of the Cape Agulhas extending to the north of Namibia (Claase, 2019). The research will contribute to the relatively small pool of South African literature on *Ecklonia maxima* and related waste.

This research will, further, contribute to knowledge on the beneficial use of *Ecklonia maxima*, which may also be generalisable to other seaweed or algae species, with a focus of reintroducing these waste residues back into the production line, and, thus, promoting the waste management hierarchy and circular economy concepts.

1.7 Structure and outline of the dissertation

The dissertation is made up of five chapters, which are structured as follows:

- **Chapter 1: Introduction**

This chapter gives the reader a brief introduction and background on seaweed processing, particularly *Ecklonia maxima*. The scope and rationale for the research are introduced. Research aims and objectives are clearly stated.

- **Chapter 2: Literature review:**

Literature review on the beneficial uses of seaweed waste residue internationally and locally, the review will also include the structural characteristics of *Ecklonia Maxima*. Chapter 2 focuses on the chemical composition of *E. maxima*, which informs the alternative uses of *E. maxima*. The advantages and disadvantages of each alternative are addressed.

- **Chapter 3: Methodology**

This chapter details the research design and methodology that was used to address the research objectives.

- **Chapter 4: Results and discussion**

The research findings and related interpretation of results are provided in Chapter 4.

- **Chapter 5: Conclusions and recommendations**

Chapter 5, ultimately, provides a conclusion to the research objectives, and recommendations are made where applicable.

1.8 Conclusion

The chapter provided a general introduction into the seaweed processing industry, particularly focusing on the use of *Ecklonia Maxima* and its resultant waste streams. The problem statement, research aim and objectives, scope, and assumptions and limitations of the research were provided. The next chapter, Chapter 2, provides an overview of international and local literature on the beneficial uses of *Ecklonia maxima* waste residue and the applicable South African legal and policy environments, to provide context to the research.

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

The main purpose of this chapter is to provide an overview of what past and present research reports on the local and international beneficial uses of spent *E. maxima*. Where information on *E. maxima* is not available, the beneficial use of other algae species is referred to. This chapter aims to address the following objectives:

- Providing information on RO1: Determining the chemical composition and characteristics of *Ecklonia maxima* waste residue; and
- Exploring RO2: Identifying the possible beneficial uses of *Ecklonia maxima* waste residue.

The literature review chapter also provides the background and context to the research, which includes a summarised production flow plan outlining the processing of *E. maxima*. It is important to note that this process description will only be brief as the detailed process is deemed to be Intellectual Property. A brief discussion on the various applicable legislative instruments for seaweed waste residue management will also be provided.

Literature review is critical to bridging the gap between historical practices/context and present developments (Boote & Beile, 2005). The literature review was performed by using platforms such as Scopus, Google Scholar and the NWU Library Repository by using keywords such as “Seaweed waste residue”, “*Ecklonia maxima*”, “*E. maxima*”, “algae waste”, “spent seaweed applications”, “alternative use”, “beneficial use”, “algae reprocessing” etc. in different combinations.

2.2 Application of seaweed in the agricultural sector

Seaweeds have been used in the agricultural sector either directly or in composted form to condition the soil, optimise plant growth and enhance performance (Craigie, 2011). In the second half of the twenty first century, it was recommended that cabbage seedlings be transplanted into a seaweed mulch that has been composted. This was one of the early applications of seaweed extracts in agriculture (Nabti *et al.*, 2009). To reduce the production of toxic sulfhydryl compounds seaweed residue was spread in the fields and turned periodically, a process they called “weathering”. This allowed the seaweed residue to aerate (Craigie, 2011). Studies show that in 1949 the first method was developed to produce a liquid seaweed product for agricultural applications (Nabti *et al.*, 2009). Seaweed extracts provide the following benefits in agriculture:

- Enhances plant growth, stress tolerance, fruit setting, root development and plant capability to fight disease;

- Improves soil structure;
- Soil water retention capacity; and
- Encourages soil microbes (Arioli *et al.*, 2015).

Seaweeds are also known to contain essential macro compounds, micro compounds, mineral elements, cytokinins, auxins, quaternary ammonium molecules and polysaccharides that are critical to plant health. Further to the aforementioned, seaweed extracts are believed to contain many other molecules that contribute to their efficacy (Arioli *et al.*, 2015).

At present, the majority of the seaweed extracts are being manufactured from the following brown seaweeds; *A. nodosum*, *Laminaria* spp., *Ecklonia maxima*, *Sargassum* spp. and *Durvillaea* spp. This research specifically focuses on *Ecklonia maxima*.

It is important to understand that seaweeds are different and as such they elicit different physico-chemical responses in plants. Most extracts range from being colourless to having a rich dark colour, their physical and chemical parameters differ vastly. The location of the seaweed and the extraction process plays a pivotal role in the efficacy of the final extract even through the seaweed material is the same (Stirk *et al.*, 2014). Due to the sensitivity surrounding methods of production, the techniques used to manufacture seaweed extracts are rarely published as they are regarded as proprietary information (Begum *et al.*, 2018).

2.2.1 *Ecklonia maxima* (Giant Kelp)

The prevalent kelp in the west coast of South Africa is *Ecklonia maxima*. This species has formed large beds in the Southern part of the Benguela region. Recent literature indicates that *E. maxima* has also spread into the Cape Agulhas region (Claase, 2019). The estimated biomass of the surface reaching kelp is 530 000 tonnes. Other kelp varieties also exist in this region but are less dominant in comparison to *E. maxima*. In the West coast region, *E. maxima* is a major component of the coastal ecosystem, and it contributes to approximately 50% of the total energy input into this ecosystem. A slight change in the distribution of *E. maxima* in this system could result in serious negative effects on the livelihoods and the coastal system, thus, sustainable harvesting and use of giant kelp is critical (Bolton *et al.*, 2012).

E. maxima is characterised by a long stipe of up to 15 metres in length and a floating head that holds the fronds at the water surface. *E. maxima* is best known as a warm temperate specie, favouring areas with an average temperature of around 21 °C (Bolton *et al.*, 2012). Giant Kelp (*E. maxima*) has been used to produce plant bio-stimulants or as a feed for abalone. The applications of this species are yet to be fully realised (Mead *et al.*, 2013).

2.3 Properties and composition of *E. maxima* waste residue

The cell walls of *E. maxima* contain proteins, minerals and polysaccharides (Lotze & Hoffman, 2016). Analytical analysis of the algae also shows a significant amount of macro elements, which include calcium, magnesium, potassium, sodium, phosphorus and sulphur; as well as relatively high concentrations of iodine (up to 223 µg/g) (Lotze & Hoffman, 2016).

The analytical results of *E. maxima* waste residue (after processing) indicating the physical characteristics as well as the elemental and heavy metal content are provided in Table 4-1, 4-2 and 4-5, respectively (SGS AFL MT20-01010: 2020; SGS Somerset West 22C C022, 2022).

The safety data sheet for *E. maxima* waste residue complies with the requirements of the Globally Harmonised System (GHS) of Classification and Labelling of Chemicals (SANS 10234, 2008) and is attached as Appendix 1. The properties of the residue are determined in terms of SANS 10234, as required by the South African *Waste Classification and Management Regulations* (GNR. 634 of August 2013). The residue is regarded as a non-hazardous organic material that is non-toxic to the environment (Company X, 2022).

2.3.1 Physical characteristics and organic matter (Proximate analysis)

Brown macroalgae are rich in polysaccharides thus rendering them an excellent source of organic matter (Thompson *et al.*, 2019). The seaweed manufacturing process (extraction process) plays a pivotal role on the composition and physical parameters of the waste stream. It stands to reason that alkaline processed seaweed will have a higher pH in comparison to acid processed seaweed (Boukhari *et al.*, 2020). The waste in question is as a result of the Cell burst technology, the physical and organic matter residue has the following parameters:

- pH: 4.0 – 4.6, this is as a result of their sterilant of choice. The product is preserved using a combination of organic acetic acid and hydrogen peroxide.
- Moisture content: ≤90%, Studies have shown that *E. maxima* contains an average of 90% moisture content (Khalil *et al.*, 2016; Rocher *et al.*, 2021). The ratio of seaweed input to the seaweed residue output is 1:1 (at the company in question).
- Dry matter: 6 – 8% (Baghel *et al.*, 2021; Khalil *et al.*, 2016).
- Carbon: 30 – 40% dry weight (SGS Somerset West C022, 2022)

2.3.2 Elemental analysis

Nitrogen, potassium and phosphorus are known macro elements that are critical to the growth and development of plants. Brown seaweeds do provide a substantial amount of these essential nutrients to plants (Circuncisão *et al.*, 2018). Brown seaweeds have high bio-adsorptive and bio-

accumulative (sponge effect) properties, this feature allows them to have a generally higher mineral content in comparison to land vegetables (plants) (Circuncisão *et al.*, 2018). Brown seaweeds contain high concentrations of sodium and iodine (Circuncisão *et al.*, 2018; Claase, 2019) (Table 2-2). The most common elements to be found in *E. maxima* are nitrogen, potassium, phosphorus, calcium, sulphur, chloride, boron, sodium, iron, copper and molybdenum. This is not an exhaustive list as there are many more elements that can be tested in brown algae (Claase, 2019). The mineral content can range from 3.5 – 44% depending on location and other contributing factors (Circuncisão *et al.*, 2018).

2.3.3 Heavy metal content

Brown marine macro algae contain high concentrations of alginic acid and sulphated polysaccharides. Suggestions have been made regarding the purpose of these polysaccharides, one of the postulated functions is for the selective adsorption of metallic ions in saline environments (Raize *et al.*, 2004). One of the applications of Kelp involve cultivating the sea-bamboo in heavy metal laden seas, this is done in a bid for the Kelp to absorb the heavy metals from the sea (purify the water) (Claase, 2019). The common heavy metal elements are selenium, cobalt, nickel, cadmium, arsenic, mercury, lead and many others.

2.3.4 Biologically active compounds

Brown macro algae also contains some biologically active compounds that are very useful in the agricultural sector, the compounds include: auxins, cytokinins, polyamines, gibberellins and ethylene. These compounds are essential for plant growth and resilience (Arioli *et al.*, 2015; Lotze & Hoffman, 2016).

2.3.4.1 Polysaccharides

A polysaccharide is an environmentally friendly (biocompatible) polymer, it is an abundant, common and affordable carbon chain. Seaweeds make excellent sources of polysaccharides, *E. maxima* is no exception (Khalil *et al.*, 2016). Seaweeds have a wide range of polysaccharides that have different properties, this difference in polysaccharide composition is influenced by different factors such as:

- Season;
- Seaweed age;
- Species; and
- Location (Claase, 2019)

There is a gap in literature for all the possible compounds found in the South African *E. maxima*. These compounds vary in concentration based on species and the factors mentioned above. *E. maxima* has the following polysaccharides: alginates, cellulose, fucoidan and laminarin (Khalil *et al.*, 2016). The active components of the polysaccharides and their corresponding monosaccharides have shown to assist in promoting antimicrobial and antifungal activity in plants. Research shows that the monomer chains obtained from these polysaccharides contribute significantly to plant growth and resilience thus sugars are an essential component in plant bio stimulants (Breda *et al.*, 2021; Torres *et al.*, 2018).

Generally brown seaweeds have a carbohydrate content of 37 – 74% and a low lignin content (Percival, 1979). The aforementioned properties make it an excellent choice for hydrolysis which in turn leads to high cellulose conversion (saccharification) into smaller carbon chains (monomer chains, reducing sugars etc) (Percival, 1979).

Cellulose is a homopolysaccharide polymer chain that is mainly composed of β -D- glucopyranose units that are held together by β -1,4- glycosic bonds. The cellulose chain is held together by the hydroxyl groups at C-3 and C-6 position, these hydroxyl groups are responsible for the hydrogen bonding. The hydroxyl groups at C-2 position mainly maintain interaction with the neighbouring glucose molecule. The hydrogen bonding within cellulose cause it to be insoluble in water and a great number of organic solvents (Percival, 1979).

Brown seaweeds also contain a generous amount of alginates and fucoidan, the main storage polysaccharide is called laminarin. Brown seaweed cell wall is predominantly alginates and it contains about 15% – 50% of the total dry weight. Alginates are linear acidic polysaccharides that have guluronic and mannuronic acids (1,4) -linkages (Charoensiddhi *et al.*, 2016; Breda *et al.*, 2021).

Fucoidan is very soluble in water, it contains esterified sulphate groups that cross-link alginates and cellulose. The monomer chains of fucoidan are l-fucose that are linked by – (1,2)- linkages. It contributes 5 – 20% dry weight of brown seaweed (Charoensiddhi *et al.*, 2016).

2.3.4.2 Proteins

Brown seaweeds have a lower protein content in comparison to green seaweed, the protein content is dependent on the season thus there are seasonal variations in protein content (Fleurence, 1999). Brown seaweeds have about 3-15% of protein (dry weight), the prevalent amino acids in brown seaweeds are glutamic acid and aspartic acid (Fleurence, 1999). Glutamic acid and aspartic acid contribute between 22 – 44% of the total amino acid content in brown seaweeds (Munda, 1977).

Amino acids are critical to the plant to growth, their functions range from growth stimulation to germination stimulation. Once amino acids are added, they are easily absorbed by the plant, this is a critical aspect in agriculture (Claase, 2019). Production of amino acids by the plant itself is an energy intensive process therefore making use of a seaweed extract that has amino acids will stimulate rapid growth and development (Munda, 1977).

2.3.4.3 Phlorotannins

In the Algae family, brown seaweeds are known to contain a rich amount of phlorotannins, this class of compounds forms part of brown seaweeds' main phenolic compounds. The concentration varies due to various factors such as location, season, salinity of the water body, sun exposure etc, in some instances these compounds contribute an average of 25% of the seaweed's dry weight (Afonso *et al.*, 2019). Phlorotannins have a polymeric structure that is composed of phloroglucinol groups that have a high number of hydroxyl groups, this distinct feature makes them soluble in water. Extraction of these phenolic compounds has shown to have a myriad of functions which include anti-inflammatory, anti-oxidant, anti-diabetic and many others (Afonso *et al.*, 2019; Claase, 2019).

2.3.4.4 Fucoxanthin

Phaeophyte's brown colour is due to the presence of the fucoxanthin pigment compound, this compound is a xanthophyll and it falls under the tetraterpenoid class. The concentration is also dependent on the location, seasonal variation, temperature etc. Studies have shown that the compound has antioxidant properties, anti-obesity and anti-diabetic, these studies were performed in vivo (Afonso *et al.*, 2019; Lomartire *et al.*, 2021).

2.3.4.5 Polyols

Mannitol (sugar alcohol) is the dominant polyol in brown seaweeds, of which the main functions include storage of carbon and alleviation of environmental stress (Claase, 2019). An average of between 10 – 20% Mannitol can be found in brown seaweeds, the presence of this compound is also affected by location, seasonal variation, species, age and age of population (Circuncisão *et al.*, 2018). The applications of mannitol are mainly with the food production sector, it can be used as a non-nutritive sweetener, a preservative, texturiser and many other applications (Afonso *et al.*, 2019; Claase, 2019).

2.3.4.6 Betaines

Betaines are a class of quaternary ammonium compounds that are found in brown seaweeds, they are mainly used within the Agricultural sector as plant bio stimulants (Claase, 2019).

2.4 South Africa legal framework related to beneficial use of waste

The National Environmental Management: Waste Act 59 of 2008 (NEM:WA) came into effect in July of 2009, with the aim of addressing the fragmentation that still existed within South Africa's waste legislation. Sections 16 and 17 of the NEM:WA place an emphasis on the waste management hierarchy, with the aim of moving waste up the value chain, away from landfilling .

The National Waste Management Strategy (NWMS) gives effect to the objectives of the NEM:WA. The 2011 version of the NWMS highlighted the importance of the waste management hierarchy, with Goal 1 focusing on the *“promotion of waste minimisation, reuse, recycling and recovery of waste”*. Likewise, Pillar 1 of the revised 2020 NWMS focuses on “Waste Minimisation” through the implementation of the waste management hierarchy. The 2022 NWMS further focuses on the importance of extended producer responsibility and the circular economy concept (DEFF, 2020).

The NEM:WA and the NWMS are supported by a legal framework of norms and standards, and regulations. Many of these are applicable to the management of algae waste residue, and its potential beneficial use, as outlined in Table 2-4.

Table 2-1: Legislation applicable to the management of algae waste residue, and its potential beneficial use, within the South African context.

Legislation	Except (paraphrased)
National Environmental Management Waste Act (59 of 2008)	The act is aimed at improving waste management practices, reducing the use of virgin material, moving waste up the value chain. It promotes the promulgation of policies that assist in the holistic integration of waste management.
National Waste Management Strategy (NWMS) (2020)	The main aim of the strategy is to fulfil the objectives of the NEM:WA. The strategy is centred around three pillars, at the centre of the strategy is the circular economy concept (DEFF, 2020).
Waste Classification and Management Regulations (GNR.634 of August 2013)	The waste classification management system makes use of prohibitions and restrictions (such as waste with high calorific value, organic waste etc), these measures in conjunction with the testing results (SANS 10234) better inform the waste generator on

Legislation	Except (paraphrased)
	the handling of the waste in question (Roos and Alberts, 2018)
Norms and Standards for the Disposal of Waste to Landfill (GNR. 636 of August 2013)	The norms and standards address the landfill classification and containment barrier design. It details the type of waste and the type of landfill that the waste must be disposed in (RSA, 2013c).
Regulations regarding the exclusion of a waste stream or a portion of a waste stream from the definition of waste (GN 715 of July 2018)	The waste exclusion regulations provided that, if the waste generator can prove the alternative use for a waste stream, then they are allowed to apply for the exclusion of the waste stream from the definition of waste provided their decision is based on the outcome of the waste classification outlining the pros of the alternative application (RSA, 2018b).
Extended Producer Responsibility (EPR), GNR. 1184 and GNR. 1187 (GN. 400 of May 2021).	The EPR regulations are based on the cradle to grave principle. The product life cycle goes beyond the point of sale and the producer is responsible for the end of life of the product (RSA, 2021).
National Norms and Standards Composting of Organic Waste (GN. 591 of June 2021)	The sole purpose of the norms and standards is to encourage the diversion of waste from landfills. The guideline provides a detailed guidance on the establishment of an organic composting facility. They also guide on the waste generator who processes an excess of ten tonnes of organic waste a day, they are permitted to operate without a Waste management license (RSA, 2021).

2.4.1 National Environmental Management Waste Act (NEM:WA) (59 of 2008)

Section 16 and Section 17 of the NEM:WA place emphasis on the waste management hierarchy, which strives to push waste up the value chain (Figure 2-2). The success of the waste management hierarchy lies in its implementation into policy, strategy and legislation (Oelofse and

Godfrey, 2008). The realisation of the circular economy requires for manufacturers to implement the waste management hierarchy. The hierarchy highlights waste disposal and treatment as a last resort thus putting emphasis of finding alternative uses of the waste (DEFF, 2020).

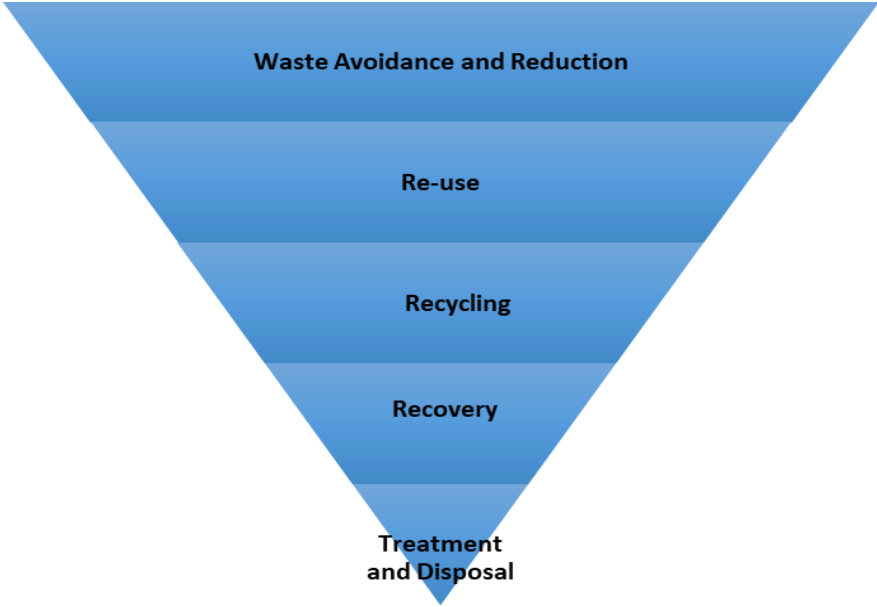


Figure 2-2: The waste management hierarchy, as proposed by the National Waste Management Strategy (Adapted from DEFF, 2020)

The hierarchy specifically advocates for the prevention, re-use, recycling, recovery and disposal of waste (Godfrey and Oelofse, 2017). Moving waste up the value chain is evident in the NWMS and a host of other command and control approaches that the government of South Africa has promulgated such as the landfill restrictions and prohibitions that forms part of *the Norms and Standards for the Disposal of Waste to Land; the Waste Exclusion Regulations; the Extended Producer Responsibility Regulations*, as well as the *Norms and Standards for Organic Waste Composting* (Table 2-1) (DEFF, 2021; Karani and Jewasikewitz, 2006).

A reduction in the waste to landfill could be achieved by diverting organic waste biomass to other potential uses, these include use as composts, solubilisation of organic biomass, agricultural soil conditioning agents etc (Makgae, 2011).

2.4.2 The National Waste Management Strategy (NWMS)

The NWMS aims to give effect to the objectives of the NEM:WA. Goal 1 of the 2011 NWMS highlighted the importance of the implementation of the waste management hierarchy, while the first pillar of the 2020 NWMS focuses explicitly on *Waste Minimisation*. Pillar 1 of the NWMS has the following focus areas:

- Creation of an environment that encourages waste minimisation- this is being achieved by the various command and control, fiscal and hybrid tools that are in place.
 - Advanced recycling fees; and
 - Waste Classification System.
- Adoption of the circular economy principles into the National Waste Management Strategy.
- Building of sustainable partnerships with all state and non-state participants.
- Minimisation of general waste streams from landfills.
- Advancement of cleaner production by introducing policies like the Extended Producer Responsibility.
- Pursue waste as a resource- emphasis being placed on the use of waste as a virgin material.
- Increase the technical capacity and innovation for beneficiation of waste as a resource- the focus has been increasing policies that support the conversion of waste to energy (Dlamini *et al.*, 2018).

The 2020 NWMS outlines the circular economy concept, which is introduced to minimise the one-way model of production and consumption (DEFF, 2020). Pillar 1 of the NWMS aims to reduce the waste that goes to landfill by 25%.

2.4.3 Circular economy

The circular economy concept reduces the pressure on natural resources by moving waste up the value chain. The 2020 NWMS explicitly focuses on the circular economy concept (RSA, 2021).

Rapid technological and scientific advancement have led to a cheaper way of production and ultimately more affordable products for consumers. Easy access to resources and products for people has caused a sudden rise in consumerism, the rise in consumerism has been characterised by a linear economy system. Resources are extracted and processed into goods that are discarded right after use without exploring the potential alternative uses of the waste (Makgae, 2011).

A sustainable holistic approach to municipal solid waste management is critical for a circular economy to be attainable. Waste resources from one industry can potentially be raw materials for another industry thus turning waste into a secondary raw material (Pires *et al.*, 2011).

2.4.4 Waste Classification and Management Regulations

The Waste Classification and Management Regulations (GN R. 634 of 2013) details the classification of waste in accordance to SANS 10234 (South Africa standard of The Global

Harmonisation System of Classification and Labelling of Chemicals (GHS)). It is a legal requirement for waste to be classified according to SANS 10234, this excludes the wastes listed in Annexure 1 of the regulations.

These properties assist in assessing the potential risks and hazards associated with the waste in question. The GHS documents details the laboratory methods of analysis for the waste, the main aim is to protect the health of people as well as to preserve the environment. This information is critical in designing a Safety Data Sheet (SDS) for the waste in question.

- Physical characteristics – corrosive to metals, flammability, oxidising nature, explosive properties etc
- Health hazards – toxicity, eye irritation, reproductive toxicity, carcinogenic properties chronic effects etc
- Environmental hazards

The Safety Data Sheet is meant to indicate whether or not a waste type is hazardous or non-hazardous. The outcome of the classification of waste determines how the waste is handled by different key role players (Nyika *et al.*, 2019).

Waste classification is an integral component of the waste management hierarchy as it assists in ensuring the protection of and wellbeing of workers, it mitigates the environmental risks through provision of product knowledge and highlights areas of potential profit maximisation (Wen *et al.*, 2013). There are two main waste classifications, it is either hazardous or non-hazardous, the global harmonised system explicitly classifies the nature of risk a waste type poses by defining it according to class, category and hazard codes.

Internationally, the adoption of the GHS has made it easier for the interpretation of similar waste stream classification from around the world. The finite details of the GHS are different country to country although the guiding principle is relatively the same. The European Union member states under the ECHA (European Chemicals Agency) have adopted the CLP (Classification, Labelling and Packaging) Regulation (EC No 1272/2008), this has its principles embedded on the United Nations' GHS. The main objective of the CLP is to ensure the protection of health as well as the environment (CIRS, 2019). The Chinese GHS is in full compliant to the United Nations GHS (CIRS, 2019).

Seaweed bio stimulants are generally classified as organic and so is their waste (Kaur, 2020). The seaweed residue that was used in the study was collected from a processing company based in Cape Town, South Africa, the company is Ecocert accredited. Ecocert is an internationally recognised organic certifying voluntary organisation (Ecocert, 2022).

2.4.5 South Africa landfill restrictions on seaweed waste residue (biomass)

The National Norms and Standards for the Disposal of Waste to Landfill (GN R. 636 of 2013) specifies the class of landfill for a particular waste stream. Landfills are classified from Class A to Class D depending on the minimum engineering design requirements for each Class, thus the norms and standards provide standard containment barrier requirements. The acceptance criteria for waste disposal to landfill is placed in four types, Type 1 to Type 4. Each particular waste type is disposed to a particular landfill Class (Mokoena, 2019; RSA, 2013c).

The disposal restrictions are based on the physical and chemical characteristics of the waste, these include but not limited to:

- Moisture content;
- pH;
- Salt/Brine content; and
- Organic matter content.

According to the prohibitions and restrictions, seaweed waste residue may not be disposed to landfill due to the high moisture content (90%) and low pH (4.0 – 4.6). The residue is regarded as having high potential for alternative or beneficial use (RSA, 2013c).

2.4.6 Regulations regarding the exclusion of a waste stream or a portion of a waste stream from the definition of waste (GN 715 of July 2018)

Waste exclusion regulations allow for the exclusion of a particular waste stream from the definition of waste, the goal is to allow for this waste stream to be recycled, reused and recovered. The sole purpose of these regulations is to reduce the pressure on virgin material and instead allow for the full realisation of raw materials, thereby, enacting the circular economy. They are also a tool meant to reduce waste that is being sent to landfill (Sutherland, 2019). In the event that the application for exclusion of the waste stream has been granted, the waste management license ceases to be a requirement instead a risk management plan becomes a requisite (RSA, 2018b)

Seaweed waste residue which is a biomass falls under the category of waste streams that one can lodge an application to the minister for exclusion of the waste stream. The potential beneficial use is subject to the composition of the waste residue as highlighted in Section 2.3.

2.4.7 Extended Producer Responsibility (EPR) Regulations (GN. 400 of May 2021)

The overarching principle in the EPR regulations is the cradle to grave principle. The scheme ensures that the financial and physical responsibility of the product lies with the producer (DEFF, 2020).

Holding manufacturers to account will aid in the transitional process as answerability is heightened within the supply chain. EPR regulations can possibly reduce the burden put on municipalities and taxpayers to manage end-of-life product fees (PlasticsSA, 2021).

2.4.8 National norms and standards composting of organic waste (GN. 591 of June 2021)

The sole mandate of the norms and standards is to provide guidance on the running and maintenance of an organic composting facility. The norms and standards also stipulate the best workable environmental preference in the composting of organic waste.

The Norms and Standards for the Assessment of Waste for Landfill Disposal (RSA, 2013b) provide a detailed breakdown of the tests that should be conducted prior to landfilling. The Norms and Standards for Disposal of Waste to Landfill focus on the type of landfill for a particular class of waste (RSA, 2013c). Organic waste residue has intrinsic economic value. Reducing the amount of organic waste disposed to landfill will assist in saving landfill airspace and reducing greenhouse gas emissions (Dlamini *et al.*, 2018).

2.4.9 Western Cape Government: Organic waste disposal to landfill restrictions

The Western Cape Government has mandated all organic waste generators to divert 50% of their organic waste from landfills by 2022, a 100% diversion from landfill is expected by 2027 (DEFF & DP, 2019). The organic waste generators are required to submit an Integrated Waste Management Plan explaining how they intend to divert 50% of their organic waste disposal to landfill. Further to the aforementioned, to assist in reducing waste disposal to landfill, the cost of landfill disposal has been increased and will continue to rise (Bungane, 2018).

2.5 International waste to landfill restrictions

Organs of state globally have put in place various legislative tools to control unsustainable waste to landfill disposal. Organic waste has become an issue of global concern, the disposal of organic waste to landfill contributes to global warming through the release of gases like methane, carbon dioxide etc at landfill sites (Weinberger *et al.*, 2019).

Below are a few countries and areas of jurisdiction and their waste to landfill restrictions. These are only illustrative examples for restrictions on certain waste streams to landfill, and an exhaustive literature review was not conducted.

2.5.1 European restrictions

The overarching landfill legislation is Directive 1999/31/EC, members of the European Union (EU) are required to have strategies targeted at reducing the amount of bio-waste that is disposed in landfill (Linden & Reichel, 2020). In 2008 the EU revised Directive 2008/98/EC, the directive made provisions targeted at reduction of waste to landfill. Directive 1999/31/EC classifies waste set for landfill according to its risk, Type 1 being inert, Type 2 is non-inert and Type 3 being hazardous waste. Directive 2002/33/EC assists in the type of landfill for each waste type and the treatment required before landfilling (Muedi, 2021). Countries in the EU have made significant progress in reducing waste disposal to landfill, significant reduction in biomass to landfill is seen in Finland, Switzerland, Germany and many others. Waste seaweed from countries like Germany and Denmark is being used in composting and as soil conditioners (Weinberger *et al.*, 2019).

2.5.2 Zimbabwean restrictions

Landfills are put into three categories, Class 1 is the Hazardous waste, Class 2 is low level hazardous waste material and Class 3 is Non-hazardous materials. The Waste and solid waste disposal regulations, statutory instrument No.6 of 2007, regulate the disposal of waste to landfill. There is nothing that directly addresses seaweed waste residue (Manyuchi & Phiri, 2013).

2.5.3 Moroccan restrictions

The main pieces of legislation linked to Biomass waste in Morocco are:

- Solid waste management (SWM) Law No. 28-00
- The National SWM program (PNM) 2008 – 2022 (Morocco, 2021)

The above pieces of legislation led to the promulgation of the Solid waste classification & Hazardous Solid waste list decree (Bensaid, 2010). Law No. 28-00, article 48 details the classification of landfills based on the type of waste they receive. Type two landfills handle the agricultural waste (Perkins *et al.*, 2014)

Waste to landfill disposal still remains an issue of global concern, the promulgation of various legislative tools has aided in bringing awareness to this crisis. Community engagement and awareness campaigns play a pivotal role in educating the masses on this issue (Bensaid, 2010).

2.5.4 The United States of America

The United States of America has effected various policies and tools to divert organic waste disposal to landfills. By 2019, 28 states had banned disposal of yard waste from landfills. These State disposal bans have caused a surge in the amount of composting facilities within the states (United States Environmental Protection Agency, 2022).

Local organic wastes or bans have also risen in certain states, Larger cities ie New York, San Fransisco, Seattle and Austin have passed ordinances prohibiting disposal of institutional and commercial organic wastes. The State of California has the AB 939 law, in 1989 this law required local jurisdictions to meet incremental organic waste diversion goals. The law requires local jurisdictions to establish integrated solid waste management planning and implementation programs (United States Environmental Protection Agency, 2022)

2.6 Alternatives uses of *Ecklonia maxima* waste residue

The National Waste Management Strategy (2020) emphasises on the waste management hierarchy and the implementation of the circular economy principles. The end goal is to maximise socioeconomic benefits while reducing the negative environmental damage (Njoku *et al.*,2019). In line with the sustainable development goals of creating jobs for all and preserving the environment, much focus has been placed on reducing wastage of resources and optimising resource consumption within the supply chain (Morocco, 2021).

Past and present studies have shown the potential that lies in seaweed waste, literature is laden with a number of potential benefits that might be applicable to *E. maxima* waste residue. Below is a list of possible beneficial uses of *E. maxima* waste residue in relation to the residue in question or other related seaweed varieties.

2.6.1 Aquaculture and animal feeds

The nature of the seaweed processing determines the other possible uses that can be explored using the spent seaweed biomass. Brown seaweeds are an excellent source of carbohydrates, proteins and vitamins (Noorjahan *et al.*, 2020). Using the laboratory reports on the composition of spent *E. maxima*, (SGS: 22C C0222, 2022), the residue would offer a nutritious source of minerals and carbohydrates in aquaculture particularly for the fish (Noorjahan *et al.*, 2020). Within the aquaculture, protein feed need is substantially high, seaweeds do contain a relatively stable amount of protein. Spent seaweed from *E. maxima* contains about 13.6% protein (Lötze & Hoffman, 2016). Using spend seaweed to feed fish is cost effective as fishfeed is highly priced

(Davidson *et al.*, 2016). Brown seaweeds have a high nutritional value and their nonanimal nature makes them appropriate for animal feed as nutraceuticals (Morais *et al.*, 2020).

Spent seaweed in aquaculture and animal feeds may have application challenges such as:

- Fish are sensitive to tannins, saponins and many others (anti nutritional factors) as such caution should be taken when administering spent seaweeds to aquatic life (Noorjahan *et al.*, 2020).
- Due to their ability to absorb inorganic compounds from the environment, relatively high concentrations of heavy metals (Morais *et al.*, 2020).
- High levels of Iodine in *E. maxima* residue, 164mg/kg (SGS, MT20-01010, 2020), Regulation (EC) No 1831/2003 elaborates on the EU member states authorisation of feed composition, (EC) No 429/2008 details the implementation of Regulation (EC) No 1831/2003 (Morais *et al.*, 2020; Circuncisão *et al.*, 2018)

2.6.2 Food sector: Source of flavourant and nutrition

For centuries production companies within the food sector have been obtaining seafood flavour from marine animals like shrimp, fish, crabs and many others (Afonso *et al.*, 2019; Laohakunjit *et al.*, 2014). Research shows that brown seaweed fortified foods are higher in fibre and minerals (Afonso *et al.*, 2019). The process of obtaining these protein hydrolysates from marine animals produces:

- poor quality flavour;
- expensive to remove excess fat during production; and
- lipid oxidation is likely to occur during the process (Laohakunjit *et al.*, 2014).

In Thailand, the process of obtaining protein hydrolysates is performed on spent seaweed after Agar extraction (Laohakunjit *et al.*, 2014).

The challenges related to the use of spent seaweed in the food sector include:

- The complexity of seaweed protein structure, ease of digestibility due to the buoyant cell-wall properties. The cell wall is made up of crystalline cellulose microfibrils, these give structure and they run parallel to the surface of the cell (Connor *et al.*, 2005).
- Low pH (acidic) results in low solubility, the current residue is between 4.0 – 4.6 pH, (SGS: 22C C0222, 2022; Fleurence, 1999).
- Selective enzymatic hydrolysis which yields better results also produces carcinogenic compounds like mono/dichloro propanols (Laohakunjit *et al.*, 2014)
- Cheaper extraction processes also yield high salt content, *E. maxima* waste residue has an average of 17659mg/L salt content (Laohakunjit *et al.*, 2014; SGS: 22C C0222, 2022)

- Food production processes are heavily regulated, Commission Directive 2008/100/EC and WHO guidelines. Adhering to all food safety standards is mandatory and quite costly (Afonso *et al.*, 2019; Circuncisão *et al.*, 2018).

2.6.3 Biogas production

E. maxima waste residue has a high polysaccharide content and is endemic thus making it an excellent choice for clean and reliable biogas production. Using biomass to produce renewable energy offers some advantages which include abundance of raw material, affordable cost and minimum interference with the environment (Darko *et al.*, 2021). Through an anaerobic digestion process *E. maxima* waste residue can be broken down (using microorganisms) to produce biogas (Darko *et al.*, 2021). Use of *E. maxima* waste residue in the production of biogas is cost effective, it also offers the advantage of preventing disposal to landfill, this can cause eutrophication in water bodies as nutrients leach from the waste residue into nearby water bodies (Santana and Encinas, 2022). To optimise the economic feasibility of biogas production, some authors suggest its production through an integrated biorefinery concept to manufacture bioproducts, biochemicals and biogas (Milledge *et al.*, 2019).

Challenges of incorporating spent seaweed in biogas production include:

- Production of biogas is a very delicate process which is dependent on pH, temperature, volatile fatty acid and the inoculum to substrate ratio (Darko *et al.*, 2021; Milledge *et al.*, 2019).
- Process set up is very costly (Milledge *et al.*, 2019)

2.6.4 Soil Conditioner/ Compost

Composting of seaweed waste residue offers the option of blending it with other organic wastes thus moving different wastes up the value chain simultaneously (Madejon *et al.*, 2021). Due to the nutritional composition of *E. maxima* waste residue (macro and micro nutrients), application of the residue as compost or as a soil conditioner offers numerous agricultural benefits. The high polysaccharide content allows the residue to enrich the soil with organic matter which is crucial to Agricultural systems (Defosse & Dellatore, 2008). In Argentina Patagonia, seaweed waste residue arising from the eutrophication of water bodies has been composted and used as a soil amendment reagent (Defosse & Dellatore, 2008). In South Africa in the Western Cape province, Reliance a composting company is paid by the majority of the seaweed processing companies to get rid of their seaweed waste residue.

Challenges of implementing spent seaweed as soil conditioners include:

- Accumulation of heavy metals in the soil, *E. maxima* residue contains a substantial amount of heavy metals (SGS: 22C C0222, 2022)

- *E. Maxima* residue has a moisture content of 92% and a very low C:N ratio thus it is prone to rapid material decomposition and Nitrogen losses (Defosse & Dellatore, 2008).
- High salinity interferes with the composting process thus composting of seaweed waste residue requires mixing with other high lignocellulose content (Defosse & Dellatore, 2008).

2.6.5 Enzymatic hydrolysis of seaweed residue for reprocessing of waste residue

Solubilisation of seaweed residue breaks down the complex carbohydrate structure to produce soluble monomer and disaccharide units. The simple sugars produced from the enzymatic hydrolysis of *E. maxima* can be reintroduced into the manufacturing system as a carbon rich raw material (Claase, 2019). This process adds the advantage of being a green technology that is not harmful to the environment and produces sustainable farming products (Zheng *et al.*, 2016)

Complex carbohydrates such as cellulose fibre are often centrifuged out of seaweed extracts and discarded as waste. Enzymatic hydrolysis of cellulose fibre offers the advantage of having a more concentrated extract that is rich in organic matter (Charoensiddhi *et al.*, 2016)

Previous studies show that solvents have been used to extract seaweed active ingredients, however enzymatic extraction offers the added advantage of it being environmentally friendly and enables the dissolution of complex carbohydrates. Further to the aforementioned, enzymatic extraction preserves the original seaweed properties, it makes use of food grade enzymes that are affordable (readily available) and have a high catalytic efficiency (Claase, 2019; Uju *et al.*, 2018).

The sole purpose of hydrolysing seaweed residue is to create a new plant bio stimulant that is rich in oligosaccharides, alternatively the hydrolysed seaweed residue can be reintroduced into the production process as a carbon enriching raw material. This in turn reduces/alleviates the pressure that is placed on virgin raw materials (Uju *et al.*, 2018; Weinberger *et al.*, 2019; Zheng *et al.*, 2016).

Challenges of using spent seaweed in enzymatic hydrolysis include:

- Enzymatic extraction is a very delicate and complex process, pre-treatment of the waste residue can be quite costly (Uju *et al.*, 2018); and
- Cost of reactor vessel installation is high (Uju *et al.*, 2018).

2.7 Factors for determining the acceptability and feasibility of beneficial uses of seaweed residue waste

The plant bio stimulant industry is fast changing thus for a company to survive in this rapidly evolving sector, there is need for research and development of the highest level. The broad

spectrum of nutritional compounds found in seaweeds make it possible for the development and improvement of existing products. Several beneficial uses of *E. maxima* waste residue may exist, but the acceptability of beneficial use options should be based on factors such as viability, feasibility, and many others (Craigie, 2011).

The study focused on the company in selecting the proximate seaweed waste residue. Production factors such as viability, demand, feasibility, cost and equipment played a pivotal role in selecting the most applicable benefit to the company (MIT Sloan Management Review, 2015).

Literature has shown that several potential uses of *E. maxima* waste residue are available for exploration. For the purposes of this research, and based on literature, the following factors have been highlighted to consider the acceptability and feasibility for the beneficial use of *E. maxima* waste residue.

- **Suitability of *E. maxima* seaweed waste residue characteristics for beneficial use:** *E. maxima* waste residue has unique chemical composition properties. Seaweeds are known for their high polysaccharide or hydrocolloid content. The seaweed waste produced by the manufacturing company in question is produced through organically accredited processes thus rendering the waste residue organic (Torres *et al.*, 2019). The residue does not contain any foreign toxic particles except the pre-existing heavy metals, seaweed is known to contain a high concentration of heavy metals and iodine (Claase, 2019). These properties/features give seaweed waste the versatility to be utilised in many sectors (Charoensiddhi *et al.*, 2017).
- **Compliance with the South African legal framework:** The suggested beneficial use should be in compliance with the South African regulatory framework (Godfrey and Oelofse, 2017).
- **Moving waste up the waste management hierarchy:** The waste management hierarchy advocates that waste disposal to landfill should be the least preferred option. Exploring the potential alternative uses of seaweed residue offers a chance to move waste up the hierarchy. Waste disposal to landfill should only be when all possible avenues have been fully considered (Bemmel and Parizeau, 2019).
- **Supporting the circular economy:** The circular economy aids in resource preservation and it optimises realisation of benefits from naturally occurring resources. The circular economy reduces pressure on resources and it support cleaner production systems (Chiarelto *et al.*, 2021).

- **Economic viability:** The scale of economically viable processing operations and valuable content in the end of life of a product play a pivotal role in its disposal or alternative use (Golev *et al.*, 2016).
- **Energy efficiency:** The rise in global warming and the promulgation of fiscal measures that ensure that manufacturing companies are energy wise and do not increase their carbon foot print makes it critical for the beneficial use option to be energy efficient (Seow and Rahimifard, 2011).
- **Business advantage and compatibility with the strategic direction of the organisation:** The case study company's main business is manufacturing of seaweed extracts that are used as plant bio stimulants for the Agricultural sector. The CEO believes in streamlining the company's core business, for the company to focus on the manufacturing of plant bio stimulants alone thus becoming the best in the field of plant bio stimulants.
- **Compatibility with existing formulations:** The end product should meet plant bio stimulant specifications alternatively the end potential benefit should be able to mix well with the current base product.
- **Practical feasibility:** the entire process flow should be viable and feasible, an easily adaptable process flow ensures that the manufacturing process is easily reproducible with minimum supervision.

2.8 Chapter summary

The literature review focused on the physico-chemical characteristics of *E. maxima* waste residue. Applicable waste legislation both locally and internationally was reviewed. The challenges and opportunities associated with the beneficial use of *E. maxima* waste residue where discussed from a business advantage point of view.

Chapter 3 discusses the methodology for the enzymatic hydrolysis of seaweed waste residue as a possible beneficial use for the company.

CHAPTER 3 METHODOLOGY

3.1 Introduction

This chapter provides a description of the research approach, the research design and the methods that were employed in addressing the research aim. The research aimed to address three research objectives, as outlined in Table 3-1. The research implemented a mixed methods approach, where data was gathered from the review of literature, as well as laboratory analysis.

3.2 Research design

The mixed methods approach utilises methods of data gathering of both qualitative and quantitative approaches into one unified study (Creswell, 2014a). For the purposes of this research, to meet the three research objectives (RO), it was critical that the researcher employed both quantitative and qualitative data gathering techniques (refer to Table 3-1). A mixed methods approach has the added advantage of allowing the research to address confirmatory and exploratory research questions thus allowing the researcher to confirm and construct theory (Creswell, 2014b). In this approach, data is either collected simultaneously or sequentially in a bid to best meet the objectives of the study (Creswell, 2014a: Creswell, 2014b). For the purposes of this research, data was collected sequentially, from RO1 to RO3, since the research objectives build on one another.

Table 3-1: Research design and data collection methods

Research objective	Proposed method	Justification
<p>1.Determining the physico-chemical composition and characteristics of <i>Ecklonia maxima</i> waste residue</p>	<p>- Waste residue samples were sent to a commercial laboratory for analysis (SGS Somerset west laboratory and SGS Agrifood laboratory)</p> <p>- The composition information from the laboratory analysis was supplemented by means of literature review.</p>	<p>- Laboratory analysis provided insight on the state and composition of <i>E. maxima</i> waste residue. the composition of the waste residue guided the researcher on identifying the most applicable beneficial use of the waste residue (RO2) (Creswell, 2014a: Creswell, 2014b)</p> <p>- Literature review provided a cost-effective way of determining the composition of seaweed. Most of the complex compounds are very costly to analyse.</p>
<p>2. Identifying the possible beneficial uses of <i>E. maxima</i> waste residue (based on the findings of RO1), and ranking these beneficial uses to determine the most preferred beneficial use</p>	<p>- Firstly, international and national literature was explored to identify (i) the possible beneficial uses of the waste residue and (ii) the criteria which should be taken into account when considering the beneficial use of <i>E. maxima</i> waste residue.</p> <p>- The potential beneficial uses were ranked based the criteria gleaned from literature to determine the most preferred beneficial use.</p>	<p>- A list of criteria was used to rank the most preferred beneficial use option. Criteria included:</p> <ul style="list-style-type: none"> • Suitability of composition for beneficial use, • Compliance with South African legal framework, • Moving waste up the waste management hierarchy • Supporting the circular economy, • Economic viability, • Energy efficiency, • Business advantage, • Compatibility with existing formulations, and • Practical feasibility.

Research objective	Proposed method	Justification
3.Determining the optimal conditions for the most preferred identified beneficial use of <i>E. maxima</i> waste residue.	- Laboratory scale in vitro trials were carried out on the most preferred beneficial use category to determine the optimal conditions under which it should take place. The outcome of the ranking process (RO2) determined that enzymatic hydrolysis were deemed to be the most preferred beneficial use option (Refer to Section 4.4 in Chapter 4).	-Laboratory experiments provide guidance on how to structure factory scale operations, this is critical in assessing the feasibility of a proposal before investing financially (Creswell, 2014a: Creswell, 2014b)

3.3 Data collection

The overview of the data collection methods used to address the research questions are outlined in Table 3-1. Data collection methods included: laboratory analysis to determine the composition of *E. Maxima* waste residue, literature review and document evaluation, as well as in vitro laboratory trials to determine the optimal conditions for the most preferred beneficial of *E. maxima* waste.

3.3.1 Laboratory analysis to determine the composition of *E. Maxima* waste residue

Two laboratories, accredited against ISO 17025 and SANAS analytical methods, SGS Somerset West and SGS Agrifood Laboratory Maitland conducted the elemental and physical analysis of the *E. maxima* waste residue. The analytical methods used by the laboratories are summarised in Table 3-2.

Table 3-2: Analytical methods used to determine the composition of *E. maxima* waste residue

Analyte	SGS Method employed
Total Nitrogen	DUMAS
Heavy metals	Aqua regia digestion (ISO standard 11466 [8] and ICP quantification
Minerals	Aqua regia digestion (ISO standard 11466 [8] and ICP quantification
Carbon	LECO, AOAC Method 972.43
Iodine	ICP – MS
Electrical Conductivity	Standard Methods for the Examination of Water and Wastewater: 2520B
pH	Standard Methods for the Examination of Water and Wastewater: 4500-H+
Dry matter	AOAC 930.15
Moisture	AOAC 930.15

Literature review supplemented the laboratory analysis, with additional information on biologically active compounds (refer to Sections 2.3.4 in Chapter 2).

3.3.2 Literature review

Research allows us to advance our mutual understanding of a subject matter, a comprehensive literature review is the prerequisite for a substantive and detailed research. The strength and weaknesses of what has been done before informing our methodology when answering the research questions. Generativity has been described as the capability to build research on what has been done by those who came before us (Boote & Beile, 2005). A comprehensive literature review is the building ground for both theoretical and methodological refinement, this in turn improves the excellence and value of subsequent research (Boote & Beile, 2005).

The literature review was conducted by a thorough investigation of relevant internet sources, these sources included peer-reviewed journals and books. The aforementioned sources were obtained through Google Scholar, Scopus, and Science Direct. Institutional publications such as dissertations and theses were obtained from the North-West University (NWU) Library website (NWU Institutional Repository: Boloka). Documents from international, national and local spheres of government were also taken into consideration.

The literature review had the following purpose:

- Give a concise understanding of the composition of *E. maxima* waste residue, this was achieved using data that was obtained in previous studies (supplementing the laboratory analysis);
- Identify the beneficial uses of *E. maxima* waste residue; and
- Determine criteria towards identifying the most preferred beneficial use.

3.3.2.1 Literature review to supplement composition information on *E. maxima* waste residue

Quantification of complex compounds is very costly and a specialised process. It requires the expertise of highly trained chemists and academic professionals. Analysis of complex compounds, such as plant growth hormones, polysaccharides, as well as proteins, phlorotannins, fucoxanthin, polyols and betaines are performed in international laboratories, which makes the process costly and highly regulated. For the purposes of this research, literature review was used for the compounds listed below:

- Plant growth hormones (auxins, cytokinins, polyamines, gibberellins), these are complex compounds that require specialised research groups to quantify. The waste generating company normally uses Palacky University (Spain) to quantify these compounds for it. The tests can range from 100 Euro to 200 Euro per sample, this is very costly. Refer to section 2.3.4.
- Polysaccharides (laminarin, fucoidan, alginates, cellulose), seaweeds have varying types of polysaccharide units. The amount of polysaccharide chains in *E. maxima* is unknown, trying to quantify all of them is a complex procedure that requires specialised skills and resources (Khalil *et al.*, 2016). Refer to section 2.3.4.1.
- Protein, phlorotannins, fucoxanthin, polyols and betaines: Although these compounds are present in *E. maxima*, their quantities are very minute and accessing them is a very complex process (Afonso *et al.*, 2019; Claase, 2019). Refer to section 2.3.4.2 – 2.3.4.6.

3.3.2.2 Literature review to identify the possible beneficial uses

A thorough understanding of *E. maxima* waste residue was found to be a prerequisite for potential beneficial uses of the waste residue. Following the establishment of the physico-chemical and compositional properties of the waste residue, an intensive international and local literature review was conducted for the potential beneficial uses (Li *et al.*, 2020). The literature review was focused on integrating outcomes and viewpoints of various practical uses to elucidate the most applicable beneficial use within South Africa and ultimately the concerned manufacturing company (Snyder, 2019).

To determine the possible beneficial uses of the waste residue, a systematic literature review was conducted. The process is as follows, Identification of problem, critical discussion of previous studies, identification of knowledge gaps, formulate research objective and literature review to address objectives respectively (Synder, 2019).

To obtain any information of potential uses of the waste residue the following search phrases were used in different combinations:

- *Brown macro algae waste residue use;*
- *Ecklonia maxima waste applications;*
- *Seaweed waste residue benefits;*
- *Seaweed processing waste;*
- *Ecklonia maxima applications; and*
- *Phaeophyta waste applications.*

This was followed by researching on the individual beneficial uses that came from the above search phrases (as outlined in Sections 2.6.1 – 2.6.5 of this dissertation). In the context of the manufacturing company and the already existing product portfolio, enzymatic hydrolysis of the waste residue was deemed to be most benefiting to the company. Enzymatic hydrolysis effectiveness was further evaluated through laboratory experiments conducted by the researcher.

Majority of the journal articles were found on Google Scholar, Scopus and the North-West University library repository. The search gave articles from around the globe, both locally and internationally. The seaweed industry is still fairly new in that there are not many articles however majority of the articles were between 2000 and 2021. Academic journals, theses, government and organisational reports, company websites, conference reports, textbooks and online articles.

3.3.2.3 Literature review to determine the criteria which should be taken into account when ranking beneficial uses for their preferability

The potential beneficial uses of *E. maxima* waste residue were further assessed based on a criteria that was informed by literature, company business goals and the National Waste Management Strategy. The potential beneficial uses included: Aquaculture & animal feeds, Food sector as a source of flavourant and nutrition, biogas production, soil conditioner, compost and enzymatic hydrolysis of the waste residue (refer to section 2.6.1 – 2.6.5). The following criteria was used to obtain the most applicable beneficial use:

- Suitability of *E. maxima* seaweed waste residue characteristics for beneficial use;
- Compliance with the South African legal framework;

- Moving waste up the waste management hierarchy;
- Supporting the circular economy;
- Economic viability;
- Energy efficiency;
- Business advantage and compatibility with the strategic direction of the organisation;
- Compatibility with existing formulations; and
- Practical feasibility (Muedi, 2020).

The preferred beneficial use was further analysed through laboratory experiments (Section 3.3.4).

3.3.3 Ranking of beneficial uses

The outcomes of the literature review informed the beneficial use options (see Section 2.6) as well as the criteria (See Section 2.7) to determine/rank the beneficial use options (Table 3-3). The most preferred beneficial use option was the option which had positive responses based on the criteria used.

Table 3-3: Ranking table/matrix used to rank the different beneficial use options for *E. maxima* waste residue, to ultimately determine the most preferred beneficial use option.

Criteria to determine preferable beneficial use	Aqua culture and animal feeds	Food sector (flavourant and nutrient)	Biogas production	Soil conditioner/ composting	Enzymatic hydrolysis for reprocessing of waste residue
Suitability of composition for beneficial use					
Compliance with South African legal framework					
Moving waste up the waste management hierarchy					
Supporting the circular economy					
Economic viability					
Energy efficiency					

Criteria to determine preferable beneficial use	Aqua culture and animal feeds	Food sector (flavourant and nutrient)	Biogas production	Soil conditioner/ composting	Enzymatic hydrolysis for reprocessing of waste residue
Business advantage and compatibility with the strategic direction of the organisation					
Compatibility with existing formulations					
Practical feasibility					

The results of the ranking are provided in Chapter 4 (Section 4.3) in more detail.

3.3.4 Determining the optimal conditions for the most preferred identified beneficial use of *E. maxima* waste residue: Enzymatic hydrolysis of *E. maxima* waste residue

The outcome of the ranking process of RO2 was that enzymatic hydrolysis of seaweed waste residue for the purposes of reprocessing the waste residue was the most preferred option (refer to the outcomes of Section 4.3 of this dissertation).

Enzymatic hydrolysis is a process where complex carbohydrates (polysaccharides) are broken down into smaller soluble monomer and disaccharide chains. These smaller monomer and disaccharide chains are soluble and easily incorporated into products/formulations where solubility is of paramount importance (Percival, 1979).

To achieve research objective 3 (i.e. determining the optimal conditions for the most preferred beneficial use, i.e. enzymatic hydrolysis) *in vitro* laboratory tests were performed based on the optimisation of the enzymatic hydrolysis of *E. maxima* waste residue. Two principal aspects were tested, namely (i) the type of enzyme and (ii) the type of pre-treatment reagent.

Based on information gleaned from literature and previous, similar studies, the enzymes and pre-treatment options outlined in Table 3-4 were tested.

Table 3-4: Enzymatic hydrolysis enzyme and pre-treatment combinations (Charoensiddhi *et al.*, 2014)

Enzyme	pH (water adjusted pH)	Enzyme Temperature °C for 24 hours	Pre-treatment: Peracetic acid 80 °C for 11 hours	Pre-treatment: 0.1% Sulphuric acid 100 °C for 1.5 hours
Celluclast® 1.5L	4.8	50	✓	✓
Viscozyme®	4.5	50	✓	✓
Ban® 480L	6.0	50	✓	✓
Viscozyme® + Celluclast® 1.5L	4.5	50	✓	✓

The two main pre-treatment reagents that were used, included (i) sulphuric acid at an inclusion rate of 0.1% (98% Sulphuric acid); and (ii) peracetic acid at an inclusion rate of 9.1% (Medichem 15% H₂O₂) (Table 3-4).

The experiment followed a two-step process: first a pre-treatment step, followed by the enzymatic hydrolysis (Uju *et al.*, 2018) as described in Section 3.3.4.1.

3.3.4.1 Method description

Enzymes were added with a slight modification (waste residue was not dried prior), substrate: enzyme ratio was 10:1 in pH adjusted water. The mixture was left at 50 °C for 24Hrs (Charoensiddhi *et al.*, 2015).

3.3.4.1.1 Controls

- For the pre-treatment step a control sample was subjected to the process for pre-treatment without the addition of pre-treatment reagent (Peracetic acid (PAA) or Sulphuric acid)
- For the enzymatic hydrolysis step, a pre-treated sample was subjected to the same conditions as a sample with an enzyme except that no enzyme was added.

3.3.4.1.2 Pre-treatment

Pre-treatment of seaweed biomass is essential for the production of a more porous biomass thus making it easier for the enzyme to reach the polysaccharides (Breda *et al.*, 2021). Literature shows that acids, salts, alkaline and surfactants have been used for the disruption of cell walls prior to solvent extraction or enzymatic hydrolysis (Breda *et al.*, 2021). Studies have shown that pre-treatment of the seaweed residue increases the yield of the simple sugars (Ge *et al.*, 2010).

In another study, pre-treatment of the seaweed residue using ionic liquids at 100°C for 30minutes gave optimal results for saccharification of polysaccharides. Ionic liquids (ILs) are organic salts that have a cation and an anion (Uju *et al.*, 2018). This study further explores the use of both ionic liquid and peracetic acid (PAA) as a pre-treatment, this pre-treatment produced 274 folds more glucose in comparison to a pre-treatment using only an ionic liquid. This was achieved by PAA pre-treatment at 80 °C for 3hrs followed by IL pre-treatment at 100 °C (Uju *et al.*, 2018).

Pre-treatment using organic acids offers the advantage of the acids being environmentally friendly and an extra source of organic nutrients. In this study, samples were pre-treated with PAA at an inclusion rate of 9.1%, 80 °C for 11 hours (Venegas *et al.*, 2013; Uju *et al.*, 2018).

In a study by Leilei in 2010, the optimum seaweed pre-treatment conditions were obtained using a sulphuric acid pre-treatment reagent at a concentration of 0.1% for 1 hour at 121°C (Ge *et al.*, 2010).

3.3.4.1.3 Enzymatic hydrolysis

The weakening of the cell wall structure in seaweeds can be achieved through treatment with enzymes, this process of cell wall disruption using enzymes is known as hydrolysis (Charoensiddhi *et al.*, 2014). Isolation of certain antioxidants (polysaccharides) was achieved by

the employment of, Celluclast, Ultraflo Max, Viscoenzyme L and a combination of Viscoenzyme and Celluclast (Charoensiddhi *et al.*, 2014). In this study the following enzymes (carbohydrases) were kindly supplied by Novoenzyme (Bagsvaerd, Denmark):

- Celluclast® 1.5L;
- Viscozyme® ; and
- Ban® 480L

In a study done by the university of Stellenbosch, extraction of the carbohydrate laminarin, Celluclast® 1.5L enzyme was used and the optimum condition were found to be 60 °C, pH of 3.0, and at 4g dry kelp/100ml. The study showed that a higher carbohydrate yield is possible at low pH levels and higher temperatures (Breda *et al.*, 2021).

3.3.4.1.4 pH variations

The residue was suspended in water adjusted pH by adding 1M H₂SO₄ or 1M NaOH (Charoensiddhi *et al.*, 2016). Carbohydrate enzymes have shown to perform better in pH controlled water than in a buffer solution, the buffer solution proved to have enzyme inhibitory effects (reduced the yield) (Breda *et al.*, 2021).

3.4 Data analysis

The next sections describe the analysis of data that was gathered on:

- *E. maxima* waste residue composition (RO1);
- Alternative beneficial uses of *E. maxima* waste residue (RO2); and
- Results from the enzymatic hydrolysis of *E. maxima* waste residue (RO3).

3.4.1 *E. maxima* waste residue composition (Literature review and SGS laboratory)

The data that was received from SGS Laboratory on seaweed waste residue was presented in tables to show the general chemical composition of the starting material. This was important in deciding the most applicable beneficial use. The complex compounds data was sourced from literature and an overview description was given for each complex compound.

3.4.2 Alternative beneficial uses of *E. maxima* waste residue (Literature review)

The data was presented in a table and the selection criteria described in Section 3.3.2. was used to eliminate the unapplicable beneficial application of the waste residue

3.4.3 Results from enzymatic hydrolysis of *E. maxima* waste residue laboratory trial

The results were presented in tables and graphs. Success of the enzymatic hydrolysis was measured by the yield in:

- Total sugar (glucose, maltose, fructose and sucrose) concentration; and
- Total soluble organic matter (TOM) concentration.

A comparison of the most effective pre-treatment reagent (26% PAA/ 0.1% Sulphuric acid) was done, this was achieved through the quantification of the following parameters found in the supernatant:

- Total sugar (Glucose, Maltose, Fructose and Sucrose) concentration; and
- Total soluble organic matter concentration.

The most preferred enzyme for the process was obtained by comparing the individual yields of each enzyme (Celluclast, Viscozyme, Ban and Celluclast+Viscozyme):

- Total sugar (Glucose, Maltose, Fructose and Sucrose) concentration; and
- Total soluble organic matter concentration.

3.5 Ethical considerations

In accordance with the North-West University (NWU, 2018), ethical considerations must be taken into account during the course of a research study. This study was pivoted on laboratory analysis and literature data, there was no human interaction with the exception of analytical chemists from third party testing institutes who were conducting the tests (no interviews or any other form of human interaction was conducted). The research proposal was submitted to the Scientific Committee of the Environmental Management of the North-West University, Potchefstroom Campus for consideration. The study was found to be in compliance to the standards as set out in the *Academic Rules for Masters and Doctoral Students at North-West University*. The research was considered to be a minimal ethical risk (Ethics number: NWU-01216-22-A9).

3.6 Methodological assumptions and limitations

The following assumptions and limitations are applicable to this research:

- The waste composition was found through proximate analysis due the high cost of analysis and South Africa not having a lot of specialised centres for complex compound analysis;

- The potential beneficial uses of the waste residue were based on what is feasible (affordability and legislation) within the South African context, this was sourced from literature;
- Acceptability of the alternative by the algae processing company and business advantage took precedence over majority of the accepting criteria for alternative uses;
- Reagents and chemicals required for analysis were sourced based on their availability and cost. Polysaccharide-specific enzymes like fucoidanases and alginases are quite difficult to access and are relatively expensive (Charoensiddhi *et al.*, 2016; Schanzenbach, 2012);
- The algae processing company's (Company X) research and development laboratory is not fully equipped thus for experiments requiring a vortex mixer, manual shaking was employed; and
- The majority of existing enzymatic hydrolysis tests (as reported in literature) are conducted on dry seaweed residue, but due to energy constraints (limiting the drying of large quantities of algae), the experiments were conducted on wet residue waste (Breda *et al.*, 2021).

3.7 Chapter summary

This chapter detailed the research design and the methodology implored in fulfilling the research objectives. The approach to data collection and analysis is also detailed together with the ethical considerations and limitations of the study.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Introduction

This research focused on exploring the alternative beneficial uses of *E. maxima* waste residue. The study utilised a combination of secondary and primary data sources as explained in Chapter 3 of this dissertation.

To summarise, the three objectives to achieve the aim of the research included:

Research objective 1: Determining the physico-chemical composition and characteristics of *Ecklonia maxima* waste residue

- Laboratory analysis was conducted to determine the physico-chemical composition of *E. maxima* waste residue, which was supported by academic literature.

Research objective 2: Identifying the possible beneficial uses of *Ecklonia maxima* waste residue (based on the findings of RO1), and ranking these beneficial uses to determine the most preferred beneficial use

- Potential beneficial uses were sourced from literature (secondary sources), and the academic literature was evaluated to inform the ranking of beneficial uses, in order to identify the most preferred beneficial use for the South African seaweed processing facility.
- The possible beneficial uses that were identified included (i) aquaculture and animal feeds; (ii) application in the food sector as a flavourant and nutrient; (iii) biogas production; (iii) application as soil conditioner or use in composting; (iv) enzymatic hydrolysis of seaweed waste residue for reprocessing of waste residue.

Research objective 3: Determining the optimal conditions of the most preferred identified beneficial use of *Ecklonia maxima* waste residue.

- Based on the ranking outcome in Research objective 2 (where enzymatic hydrolysis of seaweed waste residue for reprocessing was identified as the most preferred beneficial use), laboratory scale trials for enzymatic hydrolysis of *E. maxima* waste residue were carried out to determine the optimal conditions.

For the purposes of this research, the optimum conditions are defined as:

- The enzyme or enzyme combination that produces the highest yield of total soluble organic matter and total sugar; and
- The pre-treatment reagent that produces the highest yield of total soluble organic matter and total sugar.

This chapter outlines the results that were obtained for each of the three research objectives and the interpretation thereof. The following sections offer a detailed breakdown of the outcomes of each of the three research questions.

4.2 Physico-chemical composition and characteristics of *Ecklonia maxima* waste residue and its implications for beneficial use

Brown seaweeds are known for their high bioactive compound composition. The method of extraction plays a significant role in preservation of the bioactive compounds (Shukla *et al.*, 2021). *E. maxima* contains a mixture of complex compounds (phyto compounds/bioactive compounds) and elements that are essential in agriculture, pharmaceuticals, food sector, energy production and textile industry (Weinberger *et al.*, 2019).

As explained earlier, in this dissertation, the seaweed processing facility used for the purposes of this research, uses an aqueous extraction applying Cell burst technology, which may influence the composition and physico-chemical characteristics of the *E. maxima* waste residue in question.

The sections below will provide a discussion of the composition and characteristics of *E. maxima* waste residue (obtained through laboratory analysis and literature review) and its implications for beneficial use. The beneficial use options were identified during literature review (Refer to Chapter 2) and include potential use in (i) animal feed and aquaculture; (ii) use as a flavourant in the food sector; (iii) biogas production; (iv) use as soil conditioner or additive to compost; and (v) enzymatic hydrolysis of the waste residue to allow reprocessing of soluble organic matter and sugars.

The sections outline the physico-chemical properties and composition of the *E. maxima* waste residue (RQ1) and discuss the implications of the physico-chemical composition for alternative beneficial uses of the *E. maxima* waste residue (RQ2). The implications are mostly discussed within an international context, using requirements and limitations from the European Union (EU), the World Health Organisation (WHO) and other international organisations, considering potential export of the final product.

Compliance to South African requirements (where applicable) are reflected on in Section 4.3.2 where the beneficial use options are considered in relation to the South African legal framework.

The complex compound composition (i.e. polysaccharide and protein content) are outlined in Section 4.2.1. This information is gleaned from existing literature. Section 4.2.2 and its sub-sections reflects on the physico-chemical characteristics determined during laboratory analysis.

4.2.1 Complex compound composition (from literature review)

Exhaustive chemical analysis of the seaweed residue through laboratory analysis was not possible due to the costs associated with the analysis of some of the compounds. Compounds such as polyphenols, fucoidans and alginates require specialised research groups/laboratories to conduct such tests. These compounds are seldom tested at commercial laboratories, and for that reason the analysis of such compounds is usually very costly and is often conducted overseas. For the purposes of this research, literature was consulted to gain an understanding of the typical complex compound composition of *E. maxima*.

The cell wall of *E. maxima* has been found to contain the following broad classifications

- Minerals (discussed in Section 4.2.2 and its sub-sections);
- Polysaccharides; and
- Proteins (Claase, 2019).

The expected polysaccharide composition and protein content of *E. maxima*, and its implications on the beneficial use of the algae waste residue are discussed in Section 4.2.2.1 and Section 4.2.2.2, respectively.

4.2.1.1 Polysaccharide composition of *E. maxima* waste residue and implications for beneficial use

According to Lotze and Hoffman (2015), the following polysaccharides are typically found in *E. maxima*: Alginates, Cellulose, Fucoidan and Laminarin. These polysaccharides have various applications, which could be beneficial for use in human- and plant health (Claase, 2019). Alginates, Fucan, and Laminarins are regarded as soluble carbohydrates while sulphated polysaccharides, such as Fucoidans form part of the cell wall for brown seaweeds (Morais *et al.*, 2020; Percival, 1979). Fucoidan is the term that is given to sulphated complex carbohydrates containing fructose residues and some monosaccharide chains. *E. maxima* contains approximately 60% carbohydrate content (Cunha & Grenha, 2016; Rocher *et al.*, 2021).

Reflecting on the beneficial uses considered during this research, the presence of polysaccharides in the *E. maxima* waste may have the following implications:

- **Aquaculture and animal feed:** The polysaccharides in *E. maxima* exhibit a spectrum of biological activities and physicochemical properties that can be implemented in the pharmaceutical industry, however this requires further investigation for inclusion in animal and aqua culture feed (Cunha & Grenha, 2016).

- **Food sector (flavourant and nutrient):** Fucoïdan can be used in the food industry to produce functional foods. Alginates in the food industry are being implemented in the meat industry to make sausage casings (Cosenza *et al.*, 2017; Cunha and Grenha, 2016).
- **Biogas production:** Polysaccharides may be broken down by the use of enzymes to produce biogas (Morais *et al.*, 2020).
- **Soil conditioner/ composting:** *E. maxima* has a low C/N ratio thus making it an excellent raw material for composting. Carbon content originates from the polysaccharide composition of the waste residue (Al-Dulaimi *et al.*, 2021).
- **Enzymatic hydrolysis for reprocessing of waste residue:** Laminarins, fucoïdians and alginates can be hydrolysed using enzymes (cellulases) to release monosaccharide and disaccharide chains that can be easily used in the manufacturing industry (Cosenza *et al.*, 2017)

After an aqueous mechanical extraction of *E. maxima*, it is quite challenging to know the amount of growth promoting hormones that still remain in the waste residue, however based on the residue that remains behind it is no question that the bulk of the waste contains complex carbohydrates/ polysaccharides (Shukla *et al.*, 2021). Due to the complexity and costs associated with analysing these compounds, quantification of the exact concentrations of these compounds proved to be a challenge.

4.2.1.2 Protein content of *E. maxima* waste residue and implications for beneficial use

Brown seaweeds are known to have the least amount of protein in the algae family, ranging between 5 to 15% (Claase, 2019; Morais *et al.*, 2020; O' Brien *et al.*, 2022). Brown seaweeds are known to be sources of essential amino acids although no levels of methionine, cysteine and lysine block chains have been detected in brown seaweeds (O' Brien *et al.*, 2022). A rough estimate (though not completely accurate) for the protein content of *E. maxima* can be made by multiplying the nitrogen content (2.18%) by the Kjeldahl conversion factor of 6.25 (Kjeldahl method). This indicates that the estimated protein content for the *E. maxima* waste residue is approximately 13.6%. This value corresponds with the average protein content of brown seaweeds reported in literature. This opens opportunity for *E. maxima*'s application in the food sector as well as potentially in animal feed stocks (O' Brien *et al.*, 2022)

- **Aquaculture and animal feed:** *E. maxima* waste residue contains approximately 13.6% protein, animal and fish feed industries have been including seaweed residue as a functional ingredient in production of feeds. The presence of peptides gives the residue antioxidant activity which promote the growth of the Atlantic Salmon (O' Brien *et al.*, 2022).

- **Food sector (flavourant and nutrient):** The World Health Organisation recommends that an adult of 19 years and above should consume about 0.83 g of protein per every kilogram body weight per day (Guoyao, 2016). Inclusion of the seaweed waste residue in the food sector will aid in the required daily protein intake.
- **Biogas production:** Protein-rich substrates produces high volumes of ammonium and free ammonia from the breakdown of protein. The ammonium and ammonia are also damaging to the process of anaerobic digestion because of the toxicity to digesting bacteria therefore an acclimation period of low substrate concentrations must be factored in to allow the microbial community to adjust (Kovacs *et al.*, 2015).
- **Soil conditioner/composting:** Plant requires amino acids for their growth and fruiting. Co-composting of algae waste residue is recommended to reduce the amount of ammonium and ammonia that will be released as the protein breaks down during composting (Kovacs *et al.*, 2015).
- **Enzymatic hydrolysis for reprocessing of waste residue:** Enzymatic hydrolysis may be used to break down the polysaccharides. Non-enzymatic reactions have been found to enhance cellulose conversion to simple sugars (Hui *et al.*, 2015). Sufficient presence of soluble organic matter and sugars may provide the waste residue useful for further processing, and may reduce reliance on virgin materials (virgin algae).

It is, however, important to consider the physico-chemical composition of the *E. maxima* waste residue to determine its suitability for alternative use. Section 4.2.2 (and sub-sections) reflect on the laboratory analysis results of *E. maxima* waste and its implications for beneficial use.

4.2.2 Laboratory analysis of *E. maxima* waste residue and its implications for beneficial use

As outlined in Section 3.3.1., seaweed waste residue was sent to an accredited commercial laboratory, SGS Somerset West, for analysis. The composition results are described in the sub-sections to Section 4.2.2.

4.2.2.1 Physical characteristics

The physical characteristics *E. maxima* waste residue are outlined in Table 4-1 below.

Table 4-1: Physical characteristics and organic content of *E. maxima* waste residue (SGS Somerset West 22C C022, 2022)

Composition of waste residue	pH	Moisture %	Dry matter %	Ash %	Conductivity dS/m	Carbon %	Total organic matter %	C:N
	4.66	91.88	8.12	21.35	17.29	36.78	63.26	16.84

The results are similar to what Thompson *et al.* (2019) found on the compositional profile of brown algae, with a moisture between 70 – 90%, protein content between 8 – 23%, ash content between 10 – 15% and polysaccharide content of 40 - 60%.

4.2.1.1 Implications of physical characteristics for use in animal feeds and aquaculture

Digestibility of the seaweed in animals is a critical factor when considering animal feed. Monogastric and ruminants require a low ash content, consequently a high ash content hampers the application of seaweed as a feed (Bikker *et al.*, 2020). According to the Department of Nutrition, Research and Innovation of Rock River Laboratory Inc, ideally ash content in animal feed should be below 10% (Goesser, 2017). The *E. maxima* waste residue tested in this research had an ash content of 21.35%, which would make it unsuitable for use in animal feed (in its current form). For the seaweed to be suitable as an animal feed, an extra step, which involves washing of the residue, would have to be introduced to reduce the ash content prior to feed application (Bikker *et al.*, 2020). However, washing the algae to reduce the ash content may remove other valuable nutrients, and may increase the water demand necessary for algae processing.

It is important to characterise the nature of complex carbohydrates (reflected as total organic carbon) when considering the use of algae residue as animal feed. A study by Wageningen University, found low *in vitro* digestibility of organic matter in intact seaweed. This was attributed to nitrogen being bound or entrapped in poorly digestible carbohydrates (reflected as organic matter) within the seaweed. This finding indicates that the addition of seaweed to animal feed could be a problem for monogastric animals and better suited for ruminants (Bikker *et al.*, 2020). In addition to the above, the algae residue has a relatively low pH of 4.7 thus pH adjustment is highly recommended. Ideally, for use in animal feeds, the pH should be near neutral (pH 6.5 to 8) for ease of digestion (Bikker *et al.*, 2020).

Based on the factors discussed above, the physical characteristics of *E. maxima* make the residue unsuitable for use as an animal feed.

Considering the waste residue for use in aquaculture, the C:N of the algae residue was considered. The C:N ratio has recently been used as a tool to improve the development of bioflocs aquaculture systems (Boyde, 2018) A C:N ratio of 12:1 to 15:1 or higher is considered to be ideal. Bioflocs are a conversion of nitrogenous wastes into protein rich feed for the cultivation of fish, bioflocs are suspended particles and microorganisms made from a combination of bacteria, algae, fungi and detritus etc (Kumar *et al.*, 2020). The higher the C:N ratio, the greater the growth of bioflocs leading to ammonia nitrogen immobilisation. The *E. maxima* waste residue that was tested during this research has a C:N ratio of 18:1, thus making it suitable for aquaculture. However, the relatively low pH, again, makes it unsuitable for aquatic life pH adjustment is recommended (Boyd, 2018).

4.2.2.1.1 Implications of physical characteristics for use in the food sector (flavourant and nutrient)

The applications of certain organic waste residues in the food industry are possible due the broad spectrum of nutritional benefits that the residue possesses (Alfonso *et al.*, 2019). Alginates (polysaccharide) can be used in production of cold meats (sausage casing) for plant-based protein products, for instance. For any food application, extensive testing is recommended for parameters such as pH, TOC, ash, C:N ratio and moisture content. The relatively high moisture content (91.88%) of the *E. maxima* waste residue may limit the potential use in the food sector. A drying step or moisture reduction step would be necessary, which may make this option energy intensive and expensive (Vaquero & Hayes, 2015) and may render the use of algae residue unfeasible to utilise in food applications (Vaquero & Hayes, 2015).

4.2.2.1.2 Implications of physical characteristics for biogas production

Brown seaweeds have negligible lignin content thus making them an excellent choice for anaerobic digestion technology in bio-energy production (Thompson *et al.*, 2019; Uju *et al.*, 2018). Due to the high moisture content of *E. maxima* waste residue, wet anaerobic digestion would be encouraged (Bolong *et al.*, 2018). A favourable C:N ratio of 20 to 30: 1 is expected for optimal production of biogas during wet anaerobic digestion (Bolong *et al.*, 2018). The *E. maxima* waste residue tested during this research had a relatively low C:N ratio of 18:1, which was below the optimum range.

Additionally, the pH of the algae waste residue is too acidic (4 – 4.6) compared to the optimal pH range (around 7) for biogas production. Adjustment of the pH is highly recommended in order for it to be suitable for application (Bolong *et al.*, 2018).

In light of the above mentioned characteristics, *E. maxima* waste residue would be unsuitable for biogas production, co-digestion to rectify the pH and to amend the C:N would be necessary.

4.2.2.1.3 Implications of physical characteristics for use as soil conditioner/compost additive

The recommended C:N ratio for composting is 25 to 40:1 and a moisture content between 40% to 60% is recommended for composting (Madejon *et al.*, 2021). The high moisture content (91.88%) and relatively low C:N ratio (18:1) that are reported in Table 4-1 favours rapid material breakdown and nitrogen loss (Madejon *et al.*, 2021). Co-composting of seaweed algae, such as *E. maxima*, with materials containing a high carbon content and with a high lignin content is highly recommended in order for the residue to be suitable for composting application (Madejon *et al.*, 2021). The ideal soil pH for optimum plant growth is between 6 – 7.5, therefore, the pH of the waste residue (pH 4.66), in its current state, would be increasing soil acidity (Yang *et al.*, 2020).

4.2.1.1.4 Implications of physical characteristics for enzymatic hydrolysis and re-processing for further compound extraction

Company X could potentially benefit from re-processing soluble organic matter and sugars that are present in the algae waste residue. A carbon content of 36.78% (Table 4-1) suggests the presence of complex carbohydrates within the *E. maxima* waste residue and could produce organic nutrients and nutrient salts through degradation of the polysaccharide chains (Charoensiddhi *et al.*, 2014). The addition of carbohydrate enzymes to the waste residue can potentially result in the partial solubilisation of the residue and the release of sugars (Charoensiddhi *et al.*, 2016). The 91% moisture ratio will not inhibit enzymatic hydrolysis thus making is feasible to digest. Enzymatic hydrolysis allows for the collection of organic nutrients and nutrient salts from the *E. maxima* waste residue. These nutrients are critical to plant health and can be re-introduced into the manufacturing process for the manufacturing of a new product or to improve existing formulations (Zheng *et al.*, 2016). The optimal pH for enzymatic hydrolysis is enzyme-specific, therefore, the pH of the *E. maxima* waste residue should be adjusted to suit the optimum pH conditions for the enzyme in question. (Zheng *et al.*, 2016).

4.2.2.2 Elemental analysis (excluding heavy metals) of *E. maxima* waste residue

The mineral composition characteristics of *E. maxima* waste residue are outlined in Table 4-2.

Table 4-2: Elemental analysis (excluding heavy metals) of *E. maxima* waste residue (SGS Somerset West 22C C022, 2022)

Composition of waste residue	N %	K %	P %	Ca %	Mg %	S %	Cl ⁻ %	B (mg/kg)	Na (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Mo (mg/kg)
	2.18	5.58	0.35	1.46	0.61	0.51	3.94	251	17659	87	2.0	0.8

4.2.2.2.1 Implications of elemental composition for animal feeds and aquaculture

The value of nitrogen (2.18%) can be calculated back to protein by using the nitrogen to protein conversion factor of 6.25 (Kjeldahl method) as recommended by the Food and Agriculture Organisation Agency (FAO) (Maehre *et al.*, 2014; Vaquero & Hayes, 2015). The *E. maxima* waste residue consists of 13.63% protein, which could be useful in animal feed and aquaculture (Maehre *et al.*, 2014; Vaquero & Hayes, 2015). However, the elemental and mineral content of the *E. maxima* waste residue, may limit its application for use as an animal feed additive. Studies have shown that a high mineral content causes diarrhoea in pigs and poultry, while sheep and goats showed no negative impact in consumption of a high mineral diet (Bikker *et al.*, 2020).

Poultry have a maximum tolerable level of sodium chloride (NaCl) of 17 g/kg, while pigs can tolerate higher levels of 30 g/kg (Bikker *et al.*, 2020). *E. maxima* waste residue has a chloride value of 39.4 g/kg, which may make it suitable as a pig feed additive, but unsuitable as poultry feed.

Table 4-3 outlines the maximum tolerable levels of cattle for certain minerals, as stipulated by the United States National Research Council (NRC) (2005), as well as the levels of certain minerals detected in the *E. maxima* waste residue. The mineral content of the waste residue exceeds the maximum tolerable level (MTL) of potassium (K), sulphur (S), chlorine (Cl) and boron (B), which makes it unsuitable as a feed additive for cattle as a single component feed, it can however form part of a feed formulation.

Table 4-3: Beneficial minerals/elements with a maximum tolerable level (MTL) applicable to cattle by NRC (NRC, 2005)

Composition of waste residue and MTL	K%	P%	Ca%	Mg%	S%	Cl%	B (mg/L)	Fe (mg/L)	Cu (mg/L)	Mo (mg/L)
MTL	2	0.7	1.5	0.6	0.4	3	150	500	40	5
Levels in <i>E. maxima</i> waste residue	5.58	0.35	1.46	0.61	0.51	3.94	251	87	2.0	0.8

Based on the mineral content of the *E. maxima* waste residue, it is deemed to be unsuitable as an animal feed additive.

4.2.2.2.2 Implications of mineral composition for use in the food sector (flavourant and nutrient)

Macro algae generally have a high mineral content that may be important, at specific recommended dietary levels, to healthy human development (Vaquero *et al.*, 2015). Minerals are critical to human development as they assist in bone mineralisation through blood pressure regulation while also protecting the body from oxidative stress (Maehre *et al.*, 2014). When consumed in moderation (refer to Table 4-4 for RDA limits) sodium and potassium could assist in the electrolyte balance in humans (El-Said & El-Sikaily, 2013; Maehre *et al.*, 2014).

Table 4-4 outlines the concentration of minerals in the waste residue in comparison to the recommended daily allowance (RDA) as stipulated by the World Health Organisation. The waste residue contains a substantially high concentration of minerals. For instance, sodium is present at concentrations of 17 659 mg/L and boron at 251 mg/L, which could exceed the RDA of adults if dried, concentrated and added as a food additive (Koubova *et al.*, 2018)

Table 4-4: Comparative table RDA/AI (in accordance to WHO/FAO) for adults vs mineral composition in *E. maxima* waste residue (Koubova *et al.*, 2018; National Institute of Health, 2022)

Composition of waste residue and RDA	K	P	Ca	Mg	Na	Fe	Cu	B
RDA or AI (mg/Day)	4700 mg	700 mg	1000 mg	320 mg	1500 mg	18 mg	0.9 mg	1 – 13 mg
Levels in <i>E. maxima</i> waste residue	5.58 %	0.35%	1.46%	0.61%	17 659 mg/L	87 mg/L	2.0 mg/L	251 mg/L

*RDA- Recommended daily allowance.

*AI – Allowable intake

In conclusion, the potential use of *E. maxima* waste residue in the food sector may be possible, but could be complex to regulate given the mineral composition, and would be dependent on intensive testing and inclusion ratios of the seaweed waste residue in the final product.

4.2.2.2.3 Implications of mineral composition for biogas production

Nutritional elements such as nitrogen, phosphorus, potassium and iron are essential in the production of biogas as they provide nutrients for the bacteria during biogas production (McCarty,

1964; Nielsen *et al.*, 2020). Salts from metals such as sodium, magnesium and calcium can be inhibitory during the production of biogas, co-digestion in order to lower the salt content is, therefore, of importance (McCarty, 1964; Nielsen *et al.*, 2020).

4.2.2.2.4 Implications of mineral composition for use as soil conditioner/compost additive

The *E. maxima* waste residue analysed during this research is relatively high in nitrogen, potassium, phosphorus and other essential trace elements (Table 4-2). Application of this waste in agriculture could offer a vast number of plant growth benefits (Zheng *et al.*, 2016).

Lime (Calcium oxide/Calcium hydroxide) and plant ash are normally used to treat soil acidification. Mineral soil conditioners are mixtures used to rectify the pH of soil, these conditioners are alkaline and are rich in elements such as calcium, potassium, sodium and magnesium (Yang *et al.*, 2020). As mentioned in section 4.2.2.1.3, the pH of the algae waste residue is between 4.00 and 4.60, which does not make it ideal for use as a soil conditioner or compost additive, since it will not assist in preventing or correcting soil acidification (Yang *et al.*, 2020).

4.2.2.2.5 Implications of mineral composition for enzymatic hydrolysis and re-processing for further compound extraction

The mineral composition of the algae waste residue does not have a significant impact on the performance of enzymes during hydrolysis. Temperature, pH, enzyme concentration, substrate concentration, product concentration and activators are some of the key elements of enzymatic activity. Temperature, pH and enzyme-substrate ratio being the most critical to control, the aforementioned can be regulated through the use of technical data sheets (Cook *et al.*, 2020).

Company X plans to reprocess the products from the hydrolysed *E. maxima* waste residue into a plant bio-stimulant. It is important to understand that plant bio stimulants are different to fertilizers. Plant bio stimulants promote nutrient uptake, abiotic stress tolerance and enhance the quality of the fruit or crop (Jardin, 2015). Fertilizers are added to the soil to supply the plant with one or more of its essential nutrients ie nitrogen, potassium, phosphorus etc (Jaja & Barber, 2017). Organic matter content is the parameter of concern for the exportation of bio-stimulants, and mineral composition plays no significant role in the enzymatic hydrolysis for reprocessing of the waste residue.

4.2.2.3 Heavy metal and iodine content

The heavy metal and iodine content of *E. maxima* waste residue are outlined in Table 4-5.

Table 4-5: Heavy metals (non-beneficial elements) and iodine of *E. maxima* waste residue (SGS AFL MT20-01010: 2020; SGS Somerset West 22C C022, 2022)

Composition of waste residue	Se (mg/kg)	Co (mg/kg)	Ni (mg/kg)	Cd (mg/kg)	As (mg/kg)	Hg (mg/kg)	Pb (mg/kg)	I (mg/kg)
	<1.20	<0.10	<0.10	<0.04	<0.10	<0.36	<1.56	164

4.2.2.3.1 Implications of heavy metal and iodine composition for aquaculture and animal feeds

The laboratory analysis results of the *E. maxima* waste residue indicate a substantially high concentration of iodine (164 mg/kg). Iodine, when consumed within allowable limits, could trigger positive beneficial responses in the animals particularly in their muscle build up. The daily requirement for ruminants is 0.02 mg/kg iodine per day, and concentrations of 2.2 mg/kg or higher may lead to intoxication in ruminants (Kovac *et al.*, 2002). Furthermore, the National Research Council (USA) and the Society of Nutrition Physiology (Germany) have established the iodine intake values for food producing animals, such as pigs to be between 0.16 mg/kg (pigs) and 0.60 mg/kg (breeding sows) (European Food Safety Authority, 2013). The European Union has established a maximum tolerable limit of fish to iodine at 20 mg/L (European Food Safety authority, 2013). The iodine concentration of the *E. maxima* waste (164 mg/kg) is, therefore, considered to be too high for the algae waste residue to be used for animal feed or aquaculture in its current undiluted form.

Considering the concentration of heavy metals in the *E. maxima* waste, it should be noted that heavy metal toxicity is specie specific (Monagail *et al.*, 2018). Monogastric species, for example, require between 50 – 200 ppb of nickel, and a maximum of 50.2 mg/kg nickel inclusion in feeds is recommended (Samal & Mishra, 2011). On the other hand, for animal feeds, the maximum allowable concentration of arsenic under European Commission Regulation 2015/186 is 40 µg/g (EU, 2015), whereas a cobalt daily feed intake at a concentration of 10 mg/kg is considered safe (The European Agency for the Evaluation of Medicinal Products, 1998).

Table 4-6 outlines maximum tolerable limit for heavy metals from the United States National Research Council (NRC) and the Association of American Feed Control (AAFCO). The levels of selenium and mercury exceed the NRC MTL limits, which would make the *E. maxima* waste unsuitable as an animal feed.

Table 4-6: Maximum Tolerable Level (MTL) of heavy metals according to the US National Research Council (NRC) & Association of American Feed Control (AAFCO) and heavy metals (none beneficial) in *E. maxima* waste residue (Dai *et al.*, 2016; Deemy & Benjamin, 2019)

Maximum tolerable levels (MTL)	Se (mg/kg)	Cd (mg/kg)	As (mg/kg)	Hg (mg/kg)	Pb (mg/kg)
NRC MTL	0.3	10	30	0.2	10
AAFCO MTL		0.5	50	2	30
Levels in <i>E. maxima</i> waste residue	<1.20	<0.04	<0.10	<0.36	<1.56

*MTL: maximum tolerable limit

Table 4-7 indicates the maximum permissible limits (MPLs) of heavy metals for fish, as proposed by the European Commission (Mannzhi *et al.*, 2021). Again, the levels of selenium and mercury may be present at levels exceeding the MPL, the residue are, therefore, deemed to be unsuitable as a fish feed. These relatively high concentrations of mercury and selenium may be addressed by lowering the inclusion rate of the seaweed waste residue in applicable cases.

Table 4-7: Maximum permissible level (MPL) of heavy metal in fish feed vs heavy metals in waste residue (Mannzhi *et al.*, 2021)

Composition of waste residue and MPL	Se (mg/L)	Co (mg/L)	Ni (mg/L)	Cd (mg/L)	As (mg/L)	Hg (mg/L)	Pb (mg/L)
MPL (European Commission) (mg/kg) dry mass	0.5	1.5	1.00	2.00	4.00	0.02	5.00
Levels in <i>E. maxima</i> waste residue (mg/kg)	<1.20	<0.10	<0.10	<0.04	<0.10	<0.36	<1.56

*MPL - Maximum permissible level

4.2.2.3.2 Implications of heavy metal and iodine composition for use in the food sector (flavourant and nutrient)

Applications of the waste in the food industry are quite challenging as Phaeophyceae contains elevated levels of iodine. However, iodine could be beneficial in instances where there is a

deficiency of the element (Afonso *et al.*, 2019; Circuncisão *et al.*, 2018). Table 4-8 outlines the United States Food and Nutrition Board (FNB) at the institute of Medicine of the National Academies' recommended daily allowance (RDA) for iodine.

Table 4-8: Recommended dietary allowances (RDA) for iodine (National Institute of Health (US), 2022)

RDA according to age	Male (mcg)	Female (mcg)	Pregnancy (mcg)	Lactation (mcg)
Birth to 6 months	110	110		
7 – 12 months	130	130		
1 – 3 years	90	90		
4 – 8 years	90	90		
9 – 13 years	120	120		
14 – 18 years	150	150	220	220
19+ years	150	150	220	290

***Mcg: Microgram**

The World Health Organisation (WHO) recommends a slightly higher intake of iodine of 250 mcg for pregnant women (National Institute of Health, 2022). When compared to the limits provided in Table 4-8, to be adequate for use in the food sector, the algae residue will require blending with other raw materials to reduce the high concentration of 164 mg/kg iodine to an acceptable intake level.

Human consumption of seaweed is prevalent in Asian countries, unfortunately no limits exist to toxic metals that are allowable in seaweed foods or foodstuffs derived from seaweed. The European Commission Regulation (EC No 629/2008) has no specific thresholds for mercury, selenium and lead in seaweed foodstuffs or seaweed-derived foodstuffs (Circuncisão *et al.*, 2018). The European Commission, however, advises a concentration of cadmium at less than 3 mg per kg wet weight in foodstuffs that contain seaweed or are made from seaweed. In addition, the European Commission also published Regulation EC No. 1275/2013 for arsenic, which proposes a maximum concentration of 40 mg/kg in complementary feed and 10 mg/kg for a complete feed meal.

Even though there are no stipulated threshold concentrations in USA, the Food and Drug Administration together with the United States Environmental Protection Agency (USEPA) have been closely monitoring arsenic concentrations in seaweeds and its derived products (Circuncisão *et al.*, 2018; Tchounwou *et al.*, 2014). The USEPA has described arsenic as a group

1 carcinogen, while the International Agency for Research on Cancer has described arsenic as a Class 1 carcinogen (Monagail *et al.*, 2018).

The waste residue has potential for inclusion in the food sector, however extensive studies and research by suitably qualified personnel must be carried out. Due to the high iodine and heavy metal content great caution must be exercised.

4.2.2.3.3 Implications of heavy metal and iodine composition for biogas production

In a study conducted by Shafy and Mansour in 2014, it showed that heavy metal inhibition of biogas production could be expressed as follows: $Hg < Cd < Cr(III)$ (Shafy & Mansour, 2014). The study deemed the following concentrations as inhibiting:

- Mercury – 125mg/l;
- Cadmium – 170mg/l; and
- Chromium (III) – 775mg/l (Shafy & Mansour, 2014).

Another study conducted by Guo and peers in 2019 shows that the following concentration of heavy metals do not inhibit anaerobic digestion:

- Nickel – 0.8mg/l – 50mg/l; and
- Cadmium - 0.1 – 0.3mg/l (Guo *et al.*, 2019).

Trace amounts of heavy metals are known to stimulate the growth and activity of methanogens (Guo *et al.*, 2019). The heavy metals being reported above are not in excess thus they will not have an inhibitory effect on the production of biogas (McCarty, 1964).

Halogenated compounds have a negative impact on the production of biogas through anaerobic digestion. In a study by Nielsen *et al.* (2020), biogas production was noted to start decreasing when either potassium iodide or sodium iodide reached 0.2%, while an increase of iodine to 91% showed a decrease of biogas production by 96%. The current concentration of iodine is approximately 0.02%, which are not expected to have a significant negative impact on anaerobic digestion (Nielsen *et al.*, 2020).

4.2.2.3.4 Implications of heavy metal and iodine composition for use as soil conditioner/compost additive

Iodine has positive impact on the plants (biofortification), however, the specific response is largely dependent on the plant/crop specie. An application rate of 10 mg/kg soil is recommended for

optimum results. This concentration could be attainable if the waste residue is co-composted with other organic waste residues (Macias *et al.*, 2016).

Metal migration through leaching and runoff are common problems that occur with applying compost that has a high heavy metal content (Zhang *et al.*, 2012). Table 4-9 provides the existing heavy metal limits in Denmark, Brazil, the EU and USA for nickel, cadmium and lead (Souza *et al.*, 2018), while Table 4-10 outlines the allowable concentrations of heavy metals in soil.

Table 4-9: Heavy metal limits allowed in organic fertilisers in Brazil, USA and EU (Klinglmair & Thomsen, 2020; Souza *et al.*, 2018)

Heavy metal limits in fertilisers per country	Ni (mg/kg)	Cd (mg/kg)	Pb (mg/kg)
Denmark	25	0.7	45
Brazil	70	3	150
European Union	4 – 44	0.2 – 0.7	5 – 18
USA	-	0.1 – 0.8	1.1 – 27
Levels in <i>E.maxima</i> waste residue	<0.10	<0.04	<1.56

Table 4-10: World Health Organisation (WHO) heavy metal limits allowed soil (Zondo, 2021)

Waste composition and heavy metal limits	Se (mg/kg)	Co (mg/kg)	Ni (mg/kg)	Cd (mg/kg)	As (mg/kg)	Pb (mg/kg)
WHO limit	10	50	50	3.0	20	100
Levels of <i>E.maxima</i> in waste residue	<1.20	<0.10	<0.10	<0.04	<0.10	<1.56

The levels of heavy metals in the *E. maxima* waste residue analysed during this research does not exceed the limits provided in Table 4-9 and 4-10 and could, therefore, be suitable for use as a soil conditioner or compost additive. Iodine levels should, however, be taken into account and co-composting or blending with other organic materials may be necessary to lower the iodine concentration.

4.2.2.3.5 Implications of heavy metal composition for enzymatic hydrolysis and re-processing for further compound extraction

As mentioned earlier, Company X could potentially benefit from re-processing soluble organic matter and sugars that are present in the algae waste residue. In a study done by Wang in 2018, a concentration of 0.4 g/L of cobalt showed an increase in the conversion of cellulose to reducing sugar by 10.6%. A concentration higher than 0.4 g/L showed a decrease on the reducing sugar conversion (Wang *et al.*, 2018). The waste residue has a lower concentration of cobalt (<0.10 mg/L). For regulatory purposes, the concentration of cobalt shall not be increased during enzymatic hydrolysis and re-processing (Zondo, 2021). Enzymatic activity of cellulases is moderately inhibited in the presence of lead and nickel, while cadmium and mercury inhibited the enzymatic activity of cellulases at concentrations of 10 mM (Bagewadi & Ninnekar, 2018). The reported concentrations of heavy metals in the waste residue are all naturally occurring and are beyond the waste generator's control. The addition of cellulase will affect the soluble total organic matter, thus, the heavy metals content will have a minimum impact on enzymatic hydrolysis.

4.3 Ranking possible beneficial uses to determine the most preferred beneficial use (RO2)

Research objective 2 aims to rank the potential beneficial uses identified as alternatives for *E. maxima* waste residue to determine the most preferred beneficial use.

Table 4-11 provides the outcome of the ranking of the potential beneficial uses of *E. maxima* waste residue based on criteria, such as suitability of composition for beneficial use, compliance with South African legal framework, moving waste up the waste management hierarchy, supporting the circular economy, economic viability, energy efficiency, business advantage and compatibility with the strategic direction of the organisation, compatibility with existing formulations and practical feasibility. Cells highlighted in red determines that the potential use is unsuitable/inadequate, while green cells indicate that the use is considered to be suitable/adequate, and cells highlighted in yellow means that the beneficial use may be suitable under certain conditions.

Table 4-11: Criteria for acceptance of *E. maxima* waste residue beneficial use

Criteria to determine preferable beneficial use	Aqua culture and animal feeds	Food sector (flavourant and nutrient)	Biogas production	Soil conditioner/composting	Enzymatic hydrolysis for reprocessing of waste residue
Criteria 1: Suitability of composition for beneficial use	No	No	Yes, if co-processed with other wastes	No	Yes
Criteria 2: Compliance with South African legal framework	No	No	Yes	Yes	Yes
Criteria 3: Moving waste up the waste management hierarchy	Yes	Yes	Yes	Yes	Yes
Criteria 4: Supporting the circular economy	Yes	Yes	Yes	Yes	Yes
Criteria 5: Economic viability	No	No	No	Yes	Yes
Criteria 6: Energy efficiency	No	No	No	Yes	Yes
Criteria 7: Business advantage and compatibility with the strategic direction of the organisation	No	No	No	No	Yes
Criteria 8: Compatibility with existing formulations	No	No	No	No	Yes
Criteria 9: Practical feasibility	No	No	No	No	Yes

4.3.1 Criteria 1: Suitability of composition for beneficial use

The sub-sections to Section 4.2 of this dissertation provided an elaborate discussion on the suitability of *E. maxima* waste residue for alternative uses based on its physico-chemical characteristics and composition. The bullets below summarise the findings based on the suitability of *E. maxima* waste residue for the potential beneficial uses considered, based on the composition of the algae waste residue (Table 4-11).

- Aquaculture and animal feed:** The high ash (21.35%) content and relatively low pH, render the residue unsuitable for animal and aquaculture feed as a single feed component, inclusion in a feed formulation is highly recommended. Additionally, the high sodium chloride content (39.4 g/kg) may make is unsuitable for some animal species. The residue

also has selenium and mercury levels that exceed the allowable concentrations proposed by certain regulating bodies. In summary, the high ash content, high moisture content, low pH and metal content make the residue unsuitable for application in aqua culture and animal feeds (Bikker *et al.*, 2020; Boyd, 2018).

- **Food sector (flavourant and nutrient):** The high moisture content and relatively high mineral and metal content (exceeding recommended daily intake) make the unsuitable for application in the food sector (Vaquero & Hayes, 2015).
- **Biogas production:** The waste residue is rendered not suitable for biogas production by the low C:N ratio (18:1) ideally the C:N ratio should be in the range of 20 to 30:1. The low pH is detrimental to the survival of the anaerobic bacteria thus the pH will require adjustment before anaerobic digestion can commence. The salt content poses inhibitory effects to the production of biogas, co-digestion will have to be implemented in order to lower the salt content. The residue is, thus, in its current form, unsuitable for biogas production (Bolong *et al.*, 2018; Thompson *et al.*, 2019). Co-digestion with other waste types, could, however, make it suitable as a substrate for bio digestion.
- **Soil conditioner/composting:** The residue is unsuitable for use as a soil conditioner/ composting on its own due to the high moisture content, low C:N ratio and the low pH will increase soil acidity (Madejon *et al.*, 2021). Levels of heavy metals may also be of concern.
- **Enzymatic hydrolysis for reprocessing of waste residue:** The high total organic matter offers the probability of cellulase enzymes breaking down the insoluble organic matter to release monosaccharide and disaccharide sugar chains that are soluble and can be reintroduced into the manufacturing line. Enzymes are pH specific thus the pH of the residue does not negatively impact the performance of the enzyme, pH adjustment is a fairly simple process considering most cellulases operate at between pH 4 – pH 7. The mineral composition offers stimulatory effects to the hydrolysis of the insoluble organic matter (Bagewadi & Ninnekar, 2018; Cook *et al.*, 2020).

Enzymatic hydrolysis of the waste residue was found to be suitable for further investigation and experimental work due to the potential it has for reprocessing of soluble organic matter and sugars.

4.3.2 Criteria 2: Compliance with South African legal framework

The South African legal framework, as it relates to the beneficial use of waste, is outlined in Chapter 2 (Section 2.4.1 to Section 2.4.8) of this dissertation. In short, the South African legal framework emphasises the importance of landfill diversion and aims to implement the waste management hierarchy. Both the public and private sector are required (in terms of Section 16

and 17 of the NEMWA) to consider means of re-using, recycling and recovering waste, as preferred alternatives to landfill disposal.

It is, however, important that quality and other requirements related to the beneficial use of the waste meets South African requirements related to the application of the waste.

Table 4-12 provides the South African allowable limits for heavy metals (HM) and iodine related to beneficial uses such as food additives, soil values, and levels in ruminant feeds.

Table 4-12: Acceptable levels of heavy metals (HM) and iodine allowed in food, soil and ruminant feeds (Department of Health, 2006:114; Nkosi *et al.*, 2021; Zondo, 2021)

Waste residue composition and SA limits	Se (mg/kg)	Co (mg/kg)	Ni (mg/kg)	Cd (mg/kg)	As (mg/kg)	Hg (mg/kg)	Pb (mg/kg)	I (mg/kg)
SA -HM and Iodine allowed in food (human)	-	-	-	0.1 – 0.5	0.1 – 0.5	≤ 0.1	≤ 2mg/l	35 - 65
SA -HM and Iodine in allowed in soil	10	50	50	3.0	20.0	-	100	-
SA- HM and Iodine in ruminant feed	1.8	9	-	-	-	-	-	45
Levels in <i>E. maxima</i> waste residue	<1.20	<0.10	<0.10	<0.04	<0.10	<0.36	<1.56	164

*SA: South Africa *HM: Heavy metals

- **Aquaculture and animal feed:** In South Africa, the maximum tolerable limit for iodine intake for ruminant feed is 45 mg/kg while the residue has 164 mg/kg (Farm Feeds General Guidelines, Acts 36 of 1947). The relatively high concentration of iodine in the *E. maxima* waste renders the residue unsuitable for animal consumption as a complete feed, use of the residue as part of a formulation is recommended.
- **Food sector (flavourant and nutrient):** Allowable limits of iodine in food additives range between 35 and 65 mg/kg. Again, the levels of iodine in the *E. maxima* waste residue exceeds this limit, rendering the residue unsuitable by South African regulatory framework (Department of Health, 2018:114), it is recommended that the residue be used as part of a formulation in order to dilute the iodine concentration.

- **Biogas production:** Production of biogas from organic material is supported by the National Waste Management Strategy (NWMS) 2020. No specific South African regulations exist, which governs the composition of waste used for biogas production.
- **Soil conditioner/composting:** The National Norms and Standards for Composting of Organic Waste (GN. 591 of June 2021) allows the composting of waste. Certain waste streams (hazardous wastes) are excluded from being composted, however, algae waste (which is deemed to be an organic waste) is not excluded. No specific soil values exist for iodine content in soil. The levels of heavy metals in the *E. maxima* residue are within the legal requirements (Zondo, 2021) (Table 4-12).
- **Enzymatic hydrolysis for reprocessing of waste residue:** No specific South African regulations exist to govern the composition of *E. maxima* waste for reprocessing purposes.

The South African government has promulgated the Waste Exclusion Regulations (GN 715 of July 2018). Under this regulation, Company X is permitted to exclude *E. maxima* waste residue from the definition of waste, if the beneficial use application is approved by DFFE.

Company X is accredited by voluntary bodies such as Ecocert, the aforementioned is an organic certifying body that regulates the manufacturing and distribution of organic plant produce. In accordance to Ecocert, the waste residue produced by Company X meets all the requirements of organic waste thus it is suitable for alternative application due to the absence of harsh and persistent chemicals during its generation.

4.3.3 Criteria 3: Moving waste up the waste management hierarchy and Criteria 4: enabling a circular economy

The waste management hierarchy (refer to Figure 2-2 in Chapter 2) is accepted nationally and internationally as an instrument towards moving to more sustainable waste management practices. The National Waste Management Strategy (2020) has placed emphasis on waste avoidance, re-use, recycling, recovery and treatment as preferred alternatives to landfill disposal (DEFF, 2020). The circular economy is also outlined in the 2020 National Waste Management Strategy (NWMS), it is meant to reduce pressure on the natural resources while doing away with the linear production model. In terms of the circular economy concept, waste from one production line is treated as raw materials for another production line. This model of manufacturing reduces consumerism and pressure on resources while greening the economy (Oelofse & Nahman, 2018)

All potential beneficial use applications of *E. maxima* use considered during this research, are alternatives to waste disposal, and are regarded as mechanisms of moving waste up the waste management hierarchy.

4.3.4 Criteria 5: Economic viability

Economic viability addressed aspects of profit-making when looking into the implementation of each of the beneficial uses. Economic viability considers both economic benefits and externalities.

- **Economic benefits:** This encompasses the benefits that the beneficial use will deliver to society
- **Externalities:** These are economic impacts that directly impacts people who are not within the scope of the project (The World Bank, 2022)

All the beneficial uses (aquaculture and animal feed, food sector (flavourant and nutrient), biogas production, soil conditioner/composting and enzymatic hydrolysis for reprocessing of waste residue) could offer the following economic benefits:

- Job creation;
- Educational empowerment of the community; and
- Saving natural resources through full utilisation of raw materials (Gomes & Nobrega, 2005)

Considering the current company context and business model of Company X, only two of the alternative uses, namely use of a soil conditioner or composting and enzymatic analysis for reprocessing were regarded to be economically feasible.

Aquaculture, use as animal feed, and application of *E. maxima* waste residue in the food sector requires that the moisture content of the residue be significantly reduced. Moisture reduction is a complex cost intensive process, requiring investment in infrastructure (drying beds and ovens). Incorporating the cost of moisture reduction in the manufacturing process will in the end lead to a financial loss. Reduction of moisture is an energy intensive process thus it will increase the carbon footprint due to the use of coal-based electricity as a source of energy (Gomes & Nobrega, 2005).

Biogas production requires infrastructure and specialised reactor vessels for the anaerobic digestion and the gas collection. The installation and setting up of such a facility are costly. The residue also has to be pre-treated prior to anaerobic digestion.

Soil conditioning/ composting does not require reduction of moisture by drying (drying in reactor vessels), and co-composting can reduce the moisture content significantly. Enzymatic hydrolysis for reprocessing can be conducted with residue with a high moisture content thus saving on energy intensity related to moisture reduction. Therefore, based on cost, enzymatic hydrolysis for reprocessing and soil conditioning/composting were found to be more economically viable in comparison to the other beneficial uses.

4.3.5 Criteria 6: Energy efficiency

Globally, the manufacturing sector is responsible for about 33% of energy use and for about 38% of the carbon dioxide emissions (Apostolos *et al.*, 2013). In South Africa, 46% of the energy generated by Eskom is consumed by the manufacturing sector (Kan *et al.*, 2020).

For the ranking of the potential beneficial uses of *E. maxima* residue, the energy efficiency was assessed in terms of the nature of pre-treatment that would be required before the potential use. Drying of the residue prior to use was regarded as an energy intensive process due to the energy consumption of drying ovens. As mentioned in Section 4.3.4, *E. maxima* waste residue requires pre-treatment and drying when considered for application as feed, food or use as soil conditioner.

Use as soil conditioner/composting and enzymatic hydrolysis for reprocessing beneficial applications do not require any drying prior to processing thus these beneficial applications are considered to be more energy efficient.

4.3.6 Criteria 7: Business advantage and compatibility with the strategic direction of the organisation

The seaweed plant bio stimulant sector is highly competitive, with competition both locally and internationally. As discussed in Chapter 2 of this dissertation, biological active compounds give the seaweed extract its potency thus the most suitable potential application should be applicable in the agricultural sector and also should set the company apart from the other seaweed plant bio stimulant producers.

Enzymatic hydrolysis of the seaweed residue solubilises the algae fibre thus providing a substrate, which is high in polysaccharides to either be added to the already existing formulation or to be sold as a stand-alone product. This would allow for the company to maximise its profits, also reducing the current costs related to waste management.

The other potential beneficial uses, use as an animal feedstock, food sector application, biogas production and composting/soil conditioner will not offer a competitive edge to the business. The current business profile of company X is a specialisation in plant bio-stimulant production thus a diversification of the product portfolio with products other than plant bio-stimulants may not be beneficial. The company has an international presence thus making it compete with renowned seaweed processing companies such as Afrikelp, Kelp Blue, Acadian and others.

4.3.7 Criteria 8: Compatibility with existing formulations

The company currently manufactures a liquid plant bio-stimulant that is used by farmers around the world. The company's main mission is to be the best producer of organic liquid plant bio-stimulants, and any beneficial use of *E. maxima* waste residue must conform to the organic bio-stimulant registration requirements. The existing product portfolio comprises of plant bio-stimulants with slight variations due to target crops, regulatory environment of consumer and application season. The company holds Ecocert accreditation and thus the factory is built in a way that complies with the requirements of Ecocert for organic plant bio-stimulants.

Enzymatic hydrolysis of the residue for reprocessing would easily fall in this existing manufacturing process and are compatible with existing formulations. Other potential beneficial uses would require diversification of Company X's current processes, and the company will require in-depth research and a different target market in comparison to the existing client base (Anirudh, 2003).

Enzymatic hydrolysis for reprocessing does not require any changes to the existing product range, and offers the opportunity to rather enhance the existing products by addition of soluble organic matter to the seaweed extract. This option will ensure that the "new" plant bio-stimulant remains compatible with the old version (before addition of soluble organic matter). Thus, neither the manufacturer nor the consumer (farmer) needs to invest time and money in testing whether or not the improved formulation still has its intended potency (Anirudh, D. 2003).

4.3.8 Criteria 9: Practical feasibility

Practical feasibility considered the current size, location and layout of factory/site, as well as the technology/ machinery required and skill force/labour requirements.

- **Aquaculture and animal feed and use in the food sector:** Animal feed requires high level technical and scientific/ research professionals. A publication by the Agricultural Sector Education Training Authority in 2020 – 2021, emphasises the current gap and need for adequate skills and capacity in South Africa for the manufacturing of nutritious animal feed (Agriset, 2021). Company X currently does not employ any employees who are skilled in animal and aquaculture feed formulations. Company X is situated in Cape Town in close proximity to the City of Cape Town Sewage treatment facility. The close proximity of the sewage plant and the strong winds may cause problems of microbial contamination of the feed (Sousa, 2008). Separation of factories is highly recommended to prevent cross contamination of products, due to the current plant being small in size and with fewer plant equipment, separation of the factory is not possible (Sousa, 2008). The afore mentioned

aspects of the plant make manufacturing of the aquaculture and animal feed not practically feasible.

Furthermore, the current processing plant of Company X was designed for non- food item production. The risk assessment and critical control points in place are based on non-food items and would not comply to food manufacturing standards (Sousa, 2008).

- **Biogas production:** Biogas is produced through anaerobic digestion, which requires reactor vessels and gas pipes for the collection and storage of gas (DFFE, 2021). Company X manufactures liquid seaweed extract through a process of cell bursting, thus, the layout, equipment and design of the factory renders it practically unfeasible for biogas production. Biogas production would require the installation of specialised reactor tanks, which would also be a financial burden and is not financially viable (as discussed earlier). The company also does not have the technical expertise/ skilled force for biogas production.
- **Soil conditioner/composting:** The Norms and Standards for Composting of Organic Waste state that, an organic waste composting facility must not be constructed in a residential area (DEFF, 2021). Company X is situated in close proximity to a residential area. The current size and layout of the site of Company X is also not suitable for composting.
- **Enzymatic hydrolysis for reprocessing of waste residue:** The final product after enzymatic hydrolysis of the waste residue is liquid soluble organic matter. The current reactor vessels of Company X can be implemented for enzymatic hydrolysis. The size and location of the manufacturing plant does not interfere with the enzymatic hydrolysis of the waste. Company X does have the skilled professionals that are needed to conduct tests on the finished product and intended purpose. Enzymatic hydrolysis for reprocessing offers practical feasibility in that very few adjustments (such as installing a temperature control in the already existing reaction tanks, pH adjustment to suit enzyme requirement, etc) are required to the processing plant (Uju *et al.*, 2018).

4.3.9 Summary: Enzymatic hydrolysis for reprocessing of waste residue as the most preferred beneficial use

Enzymatic hydrolysis with solubilisation of the waste residue has the potential benefits of producing soluble organic matter which is essential for plant growth. Use of enzymes for the solubilisation of the waste residue is acceptable in organic farming thus this process is approved by Ecocert for the production/enhancement of organic plant bio-stimulants.

At present to increase the organic matter of the seaweed extract the company has been relying on procuring organically certified organic matter enriching reagents, this is a costly process.

Enzymatic hydrolysis of the waste offers a cost-effective method of producing organic matter. Enzymatic hydrolysis of waste for reprocessing is the only one of the considered alternative uses meeting all of the criteria outlined in Table 4-11.

- **Suitability of composition for beneficial use:** The composition and physico-chemical attributes of the waste residue were found to not interfere with the hydrolysis of the waste residue using digestive enzymes. Very few to minor adjustments (such as pH-, temperature- and viscosity adjustments) were made for the residue to be fully suitable to enzymatic hydrolysis.
- **Compliance with South African legal framework:** No restrictions exist within the current South African legal framework for the enzymatic hydrolysis and reprocessing of *E. maxima* waste residue. The enzymatic hydrolysis and reprocessing of waste are aligned with the waste management hierarchy, diverting waste away from landfilling.
- **Supporting the circular economy:** Disposing the waste residue to landfill would be supporting the linear economy however reusing the hydrolysed residue in the production system would be supporting the circular economy. This reduces consumerism thereby reducing pressure on natural resources.
- **Economic viability:** Enzymatic hydrolysis and reprocessing of *E. maxima* has both positive externalities and economic benefits. Jobs are created, pollution is reduced, and less pressure are placed on the use of natural (virgin) resources.
- **Energy efficiency:** The process is less energy intense in comparison to the other proposed beneficial applications, which require pre-treatment and drying.
- **Business advantage and compatibility with the strategic direction of the organisation:** The end-product of enzymatic hydrolysis and reprocessing is still regarded as a plant bio stimulant, this product is endorsed by Ecocert, the organic certifying body for organic farming.
- **Compatibility with existing formulations:** After enzymatic hydrolysis the soluble organic matter can be used in organic farming as a stand-alone product or it can be reformulated into the parent formulation to increase the total organic matter content.
- **Practical feasibility:** The existing labour force and the plant machinery can be easily adapted for enzymatic hydrolysis without incurring excessive costs.

Enzymatic hydrolysis was found to meet all the criteria and thus it was set aside for further investigation. Section 4.4 outlines the analysis performed to determine the optimal conditions for enzymatic hydrolysis and reprocessing of the waste.

4.4 Determining the optimal conditions for enzymatic hydrolysis for reprocessing of *Ecklonia maxima* waste residue (RO3)

Research objective 3 aims at determining the optimal conditions of the most preferred identified beneficial use of *Ecklonia maxima* waste residue.

Based on the outcome of Research objective 2, enzymatic hydrolysis of seaweed waste residue for reprocessing was identified as the most preferred beneficial use (see summary in Section 4.3.9 above).

Laboratory scale trials for enzymatic hydrolysis of *E. maxima* waste residue were carried out to determine the optimal conditions. As mentioned earlier, for the purposes of this research, the optimum conditions are defined as:

- The enzyme or enzyme combination that produces the highest yield of total soluble organic matter and total sugar; and
- The pre-treatment reagent that produces the highest yield of total soluble organic matter and total sugar.

As explained in Section 3.3.4 in Chapter 3, to determine the optimum conditions for enzymatic hydrolysis, two principal aspects were tested, namely (i) the type of enzyme and (ii) the type of pre-treatment reagent required.

The yield of the enzymatic hydrolysis process was determined by the sugar and total organic matter yield. Seaweed residue is mainly composed of polysaccharides in the form of cellulose thus the addition of enzymes causes a breaking of the C-C links releasing individual sugar molecules while also solubilising the cellulose (total organic matter). Sugar and solubilised organic matter (TOM) increase the organic matter/carbon content of plant bio stimulants, a high carbon content is normally a regulatory requirement for easy exportation of the final product to different parts of the world.

4.4.1 Pre-treatment results

Figure 4-1 shows the total organic matter in the supernatant following pre-treatment with 0.1% sulphuric acid and 26% peracetic acid (PAA).

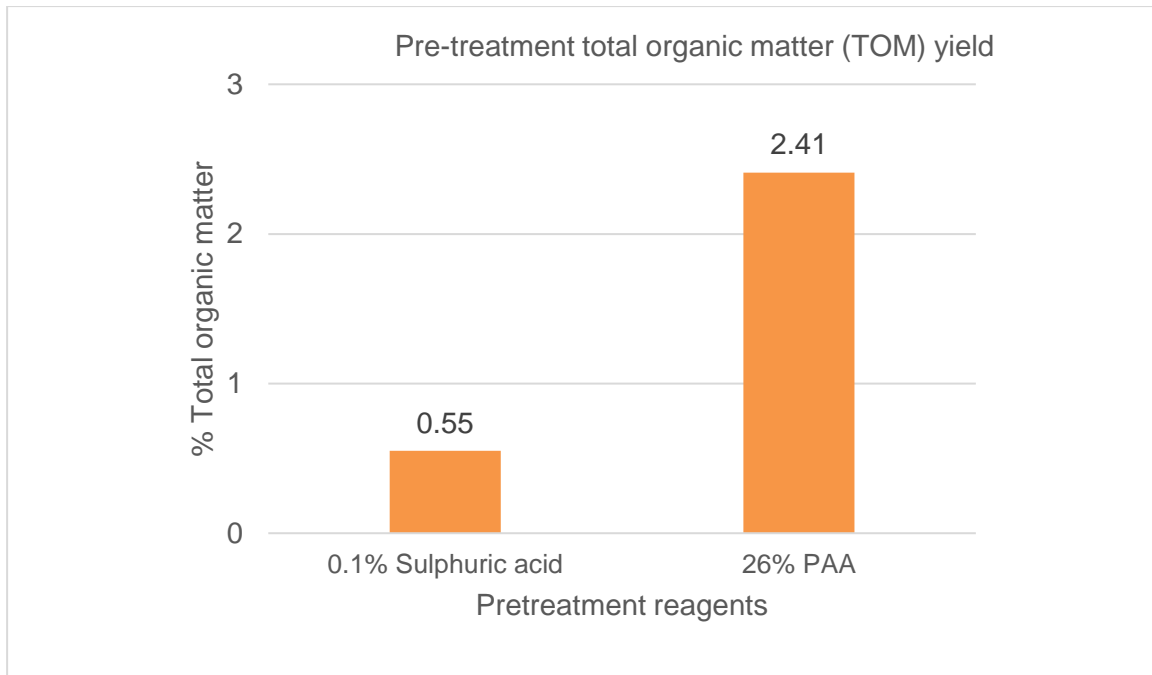


Figure 4-1: Pre-treatment supernatant total organic matter (TOM) yield results

The main aim of pre-treatment is to increase the surface area to volume ratio of the cellulose prior to enzymatic hydrolysis. Pre-treatment increases makes the cellulose more porous while also decreasing cellulose crystallinity (Uju *et al.*, 2018). The supernatant after pre-treatment with peracetic acid shows high total organic matter content compared to the 0.1% sulphuric acid pre-treatment. The supernatant of PAA (Peracetic acid) yields 4.4 times more TOM (total organic matter), which indicates that PAA allows for hydrolysis of cellulose to some extent. In a study by Uju *et al.* (2018), the hydrolysis of cellulose using PAA was also noted in a trial where it was combined with ionic liquids.

Figure 4-2 shows a comparison of pre-treatment supernatant sugar yield results after pre-treatment with 0.1% sulphuric acid and 26% peracetic acid (PAA).

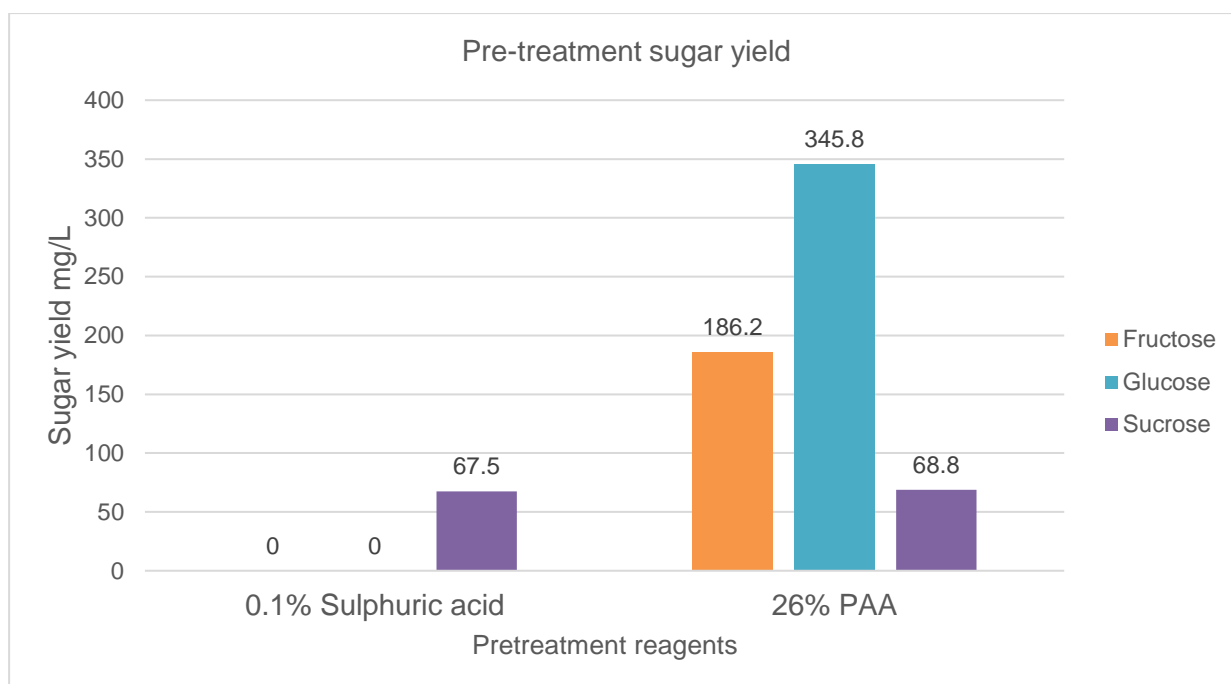


Figure 4-2: Pre-treatment supernatant sugar yield results

The supernatant was also screened for fructose, glucose, sucrose and maltose (Figure 4-2). The supernatant after the addition of 0.1% sulphuric acid only yielded sucrose, while the PAA supernatant had both reducing and non-reducing sugars (fructose, glucose and sucrose). Both supernatants yielded approximately the same amount of sucrose. Glucose was released at a higher concentration in comparison to the other sugars (for the 26% PAA treatment).

In a study done by Corredor, *et al.*, 2007, they mention the direct relation between the acid strength and the enzymatic hydrolysis yield. The higher the concentration of the pre-treatment acid the higher the concentration of the TOM in the supernatant (Corredor, *et al.*, 2007). In this study the PAA was more acidic (pH of 2) in comparison to 0.1% sulphuric acid (pH of 6).

Some studies show that hydrolysis of cellulose can be done purely using organic acids, ionic liquids and bases. One such study where no enzymes were employed for enzymatic hydrolysis, waste seaweed residue was treated with PAA first, followed by ionic liquids (Uju *et al.*, 2018). Unfortunately, literature also shows that pre-treatment of cellulose, although effective in regenerating cellulose by increasing the surface area to volume ratio, is very costly. The high temperature and pressure cause the high metallurgy costs associated with pre-treatment (Aden and Foust, 2009).

4.4.2 Enzymatic hydrolysis

As explained in Section 3.3.4 of this dissertation, the pre-treated residue was subjected to enzymes to determine the optimal TOM and sugar yields.

4.4.2.1 Total organic matter (TOM) yield

Figure 4-3 shows the comparison between the TOM yield for PAA treated residue vs sulphuric acid pre-treated for each enzyme, or enzyme combination.

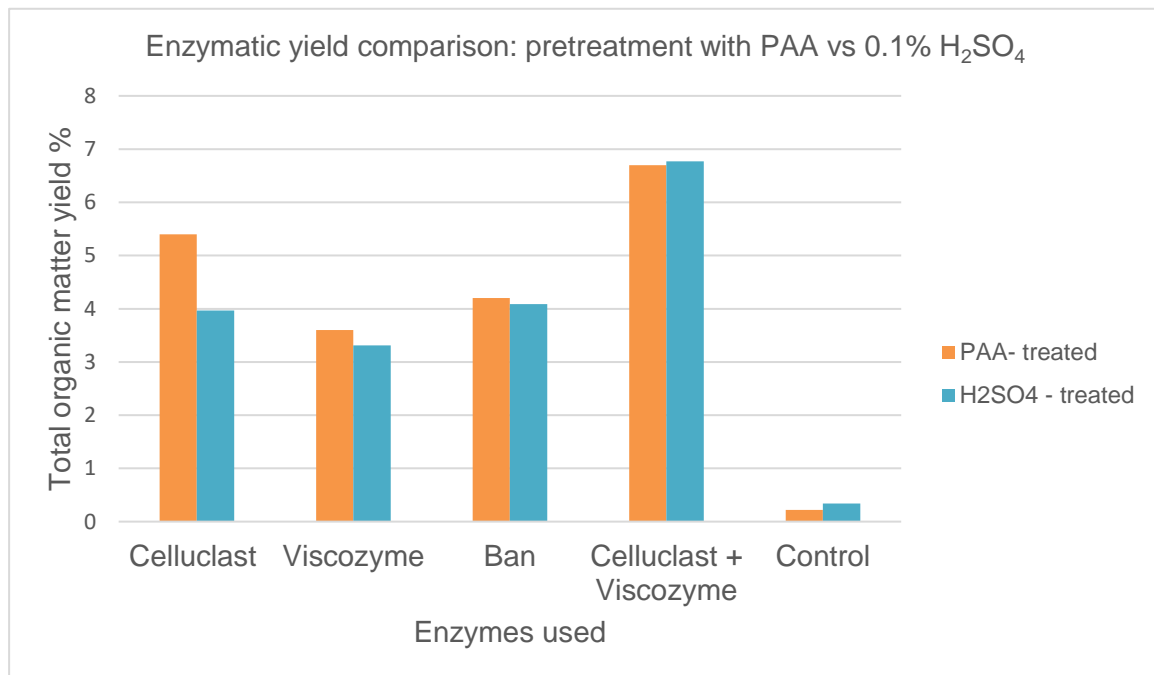


Figure 4-3: Enzymatic yield (TOM) comparison, pre-treatment with PAA vs 0.1% H₂SO₄

After being pre-treated with sulphuric acid or PAA, the seaweed residue was subjected to enzymatic hydrolysis. A control sample with pre-treated residue but excluding enzymes was included in the trial.

All three enzymes produced a substantial amount of soluble total organic matter (between 3.5 and 5.3%). A combination of Celluclast + Viscozyme produced about twice the amount of soluble TOM than the average TOM yield produced by individual enzymes.

The total organic matter (TOM) yield seems to be independent of the pre-treatment method used, with each enzyme producing roughly the same TOM yield for PAA and sulphuric acid pre-treated tests. The exception is the Celluclast enzyme, where the residue pre-treated with PAA produced

a slightly higher amount of TOM, when compared to the sulphuric acid treated residue (Figure 4-3).

4.4.2.2 Sugar yield

Figure 4-4 shows the total sugar yield (fructose, glucose, sucrose and maltose), while Table 4-13 provides the individual sugar yield at different pre-treatment and enzyme combinations.

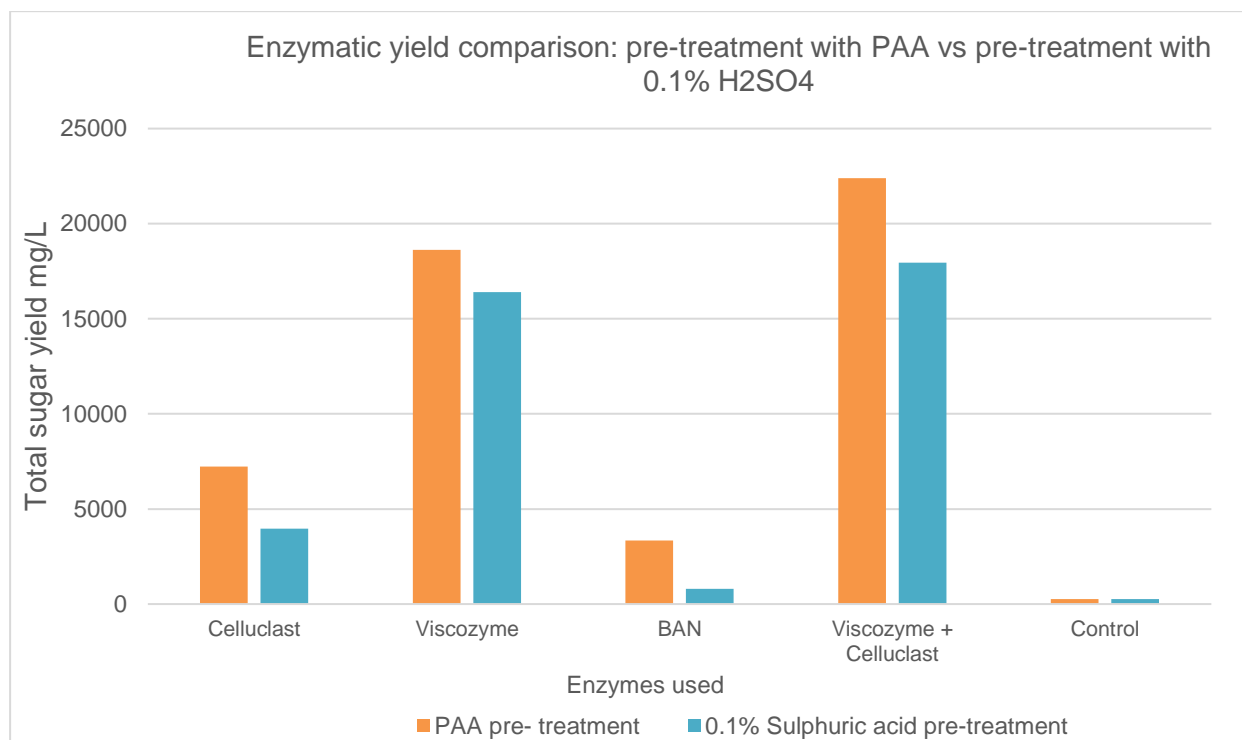


Figure 4-4: Enzymatic yield (Total sugar yield) comparison, pre-treatment with 26% PAA vs 0.1% sulphuric acid

Table 4-13: Sugar profile yield comparison showing impact of pre-treatment on yield

Enzymes	Fructose (mg/L)		Glucose (mg/L)		Sucrose (mg/L)		Maltose (mg/L)	
	0.1% H ₂ SO ₄	26% PAA	0.1% H ₂ SO ₄	26% PAA	0.1% H ₂ SO ₄	26% PAA	0.1% H ₂ SO ₄	26% PAA
Celluclast	375.2	193.5	3584.8	688.5	0	127.8	0	18.7
Viscozyme	6754.5	6827.7	9467.8	11200	186.1	154.7	0	445.6
Ban	552.1	674.3	138.1	219.1	105.3	0	0	2441.8

Enzymes	Fructose (mg/L)		Glucose (mg/L)		Sucrose (mg/L)		Maltose (mg/L)	
	0.1% H ₂ SO ₄	26% PAA	0.1% H ₂ SO ₄	26% PAA	0.1% H ₂ SO ₄	26% PAA	0.1% H ₂ SO ₄	26% PAA
Celluclast + Viscozyme	6656.3	7020	10076.4	15188.6	1223.6	183.4	0	0

Similar to the TOM yield, both the PAA and sulphuric acid pre-treated residues showed similar trends, with PAA-treated residues yielding slightly higher concentrations of total sugars (Figure 4-4).

The 0.1% H₂SO₄ treated residue exposed to Celluclast only yielded fructose and glucose (no sucrose or maltose). Residues exposed to Viscozyme, Ban and Celluclast + Viscozyme did not yield any maltose, while glucose was the common sugar in all three enzymes and it was released at higher concentrations in comparison to the other sugars.

The PAA treated residues generally yielded all sugars at the different enzyme treatments. However, residues exposed to Ban did not yield any sucrose, and residues exposed to Celluclast + Viscozyme did not yield any maltose.

PAA-pretreated residues generally yielded higher sugar concentrations than sulphuric acid pre-treated residues.

The highest concentration of total sugars was measured in residues that were pre-treated with PAA and exposed to the Viscozyme + Celluclast combination of enzymes. Sucrose was the only sugar which were present in higher concentrations in the sulphuric acid pre-treated residue than in the PAA-treated residue. When considering individual enzymes, Viscozyme yielded the highest concentrations of sugars for both PAA and sulphuric acid pre-treated residues.

In a study for extraction of antioxidants and phlorotannins from seaweed, a similar trend of Viscozyme having higher yields was observed (Charoensiddhi *et al.*, 2014). In some studies, however, the activity of Celluclast and Viscozyme is comparable, thus it is important to note the impact that these two enzymes have in breaking down cellulose (Charoensiddhi *et al.*, 2014). In the research by Charoensiddhi *et al.* (2014), Viscozyme produced an average yield of 8.5 g glucose per 100 g in pH 4.5 adjusted water, Celluclast produced 9 g glucose per 100g, and Celluclast +Viscozyme produced 10.5 g glucose per 100 g. These yields are higher than what was obtained in the current study. The only difference between these two studies is the nature of the starting material. Charoensiddhi started with a freeze-dried sample while this study was conducted on wet seaweed waste residue (90% moisture content) (Charoensiddhi *et al.*, 2014).

The reducing sugar (fructose, glucose, sucrose and maltose) results of this study do not show a pattern on the effect of pre-treatment on the sugar yield. A clear distinction, however, is in the sugar yield of each particular enzyme. The carbohydrase Celluclast is showing good performance in the total amount of solubilised organic matter that is being produced at 5 to 6%. Celluclast, however, has a lower yield for sugars in comparison to Viscozyme (carbohydrase). In a study at the University of Stellenbosch, Celluclast was said to be among the top performing carbohydrases by yielding 40% solubilisation of *Ecklonia Cava* and a high glucose yield in *Ecklonia radiata* (Breda *et al.*, 2021).

Viscozyme produced sugar yields that are very close to the yields produced by the combination of Celluclast + Viscozyme. Using the combination of enzymes might not be commercially viable, because of the cost of the enzymes. However, it is beneficial to explore optimisation of Viscozyme performance.

In summary, the test results indicate the PAA pre-treated residues generally yielded higher TOM and sugar concentrations. The combination of Celluclast + Viscozyme yielded the highest concentration of TOM and sugars, and are deemed to be the most effective enzyme combination for enzymatic hydrolysis for the purposes of reprocessing TOM and sugars.

4.5 Chapter summary

Research objective 1: Determining the physico-chemical composition and characteristics of *Ecklonia maxima* waste residue

The results of research objective 1 have shown the potential that lies in *E. maxima* waste residue. the waste residue has shown to contain a substantial amount of nutrients that can still be utilised.

Research objective 2: Identifying the possible beneficial uses of *Ecklonia maxima* waste residue (based on the findings of RO1), and ranking these beneficial uses to determine the most preferred beneficial use

A number of potential beneficial uses were gleaned from literature, these included:

- Aquaculture and animal feed;
- Food sector (flavourant and nutrient);
- Biogas production ;
- Soil conditioner/composting ; and
- Enzymatic hydrolysis for reprocessing of waste residue.

Based on the ranking criteria (Table 4-11), enzymatic hydrolysis of the waste residue for reprocessing met all the requirements of the criteria and was set aside for further experimental investigation.

Research objective 3: Determining the optimal conditions of the most preferred identified beneficial use of *Ecklonia maxima* waste residue

Total organic matter and total sugar concentrations were used to measure the success of the enzymatic hydrolysis process. Pre-treatment of the residue prior to enzymatic hydrolysis was found to be of paramount importance. PAA pre-treated residues generally yielded higher concentrations of TOM and sugars, however further investigation is still required to establish the most effective pre-treatment reagent, the required concentration and length of incubation. The combination of Celluclast + Viscozyme yielded the highest concentration of TOM and sugars.

Enzymatic hydrolysis of the waste residue requires further investigation to optimise the process, great potential lies within the process.

CHAPTER 5 CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

The aim of this research was to identify the possible beneficial uses of *E. maxima* waste residue. The aim was achieved through a mixed-methods approach, which employed laboratory analysis and literature review. Section 5.2 below provides the conclusions for each of the three research objectives, while Section 5.3 outlines practical recommendations applicable to the research scope and recommendations for further research.

5.2 Conclusions

Each of the research objectives are concluded individually in the sub-sections to Section 5.2, below.

5.2.1 Conclusions related to RO 1: Determining the physico-chemical composition and characteristics of *Ecklonia maxima* waste residue

It is important to understand the physico-chemical composition of the *E. maxima* waste residue, because it determines the suitability of the residue for different beneficial uses. The physico-chemical composition of the waste residue was determined by laboratory analysis, and supported by literature review.

The high moisture content (91.88%) and the relatively low pH (4.66) of the waste residue make it unsuitable for landfilling (in its current form), according to the prohibitions of the *Norms and Standards for the Disposal of Waste to Land* (GNR. 636 of 2013) (RSA, 2013c). On the other hand, the nutritional content of the algae waste residue makes it suitable for re-use/beneficiation.

The high organic matter content (63.26%) in the form of cellulose, together with the mineral composition of the waste residue proves the potential that lies in secondary use of the residue instead of disposal to landfill (Zheng *et al.*, 2016; Rocher *et al.*, 2021).

Elements of potential concern present in the *E. maxima* waste residue include high levels of iodine, and heavy metals, such as selenium, cadmium, arsenic, mercury and lead. Beneficial uses of the waste residue in applications with animal or human consumption should be carefully considered (Circuncisão *et al.*, 2018). The use of proximate analysis for some functional groups like fucoidans, hormones, quaternary compounds and many others could, however, potentially hinder the full realisation of the actual composition of the waste residue.

Based on the physico-chemical composition of the waste, possible beneficial uses were explored as part of research objective 2.

5.2.2 Conclusions related to RO 2: Identifying the possible beneficial uses of *Ecklonia maxima* waste residue (based on the findings of RO1), and ranking these beneficial uses to determine the most preferred beneficial use

Based on alternative uses of brown algae elsewhere in the world, the following potential beneficial uses were identified from literature:

- Animal feeds and aquaculture;
- Food sector (flavourant and nutrient);
- Biogas production;
- Soil conditioner/composting; and
- Enzymatic hydrolysis for reprocessing of waste residue.

Based on the physico-chemical composition of the waste, with the precautionary principle in mind, the *E. maxima* waste residue (in its current, unmixed/undiluted/undried form) was regarded as suitable for enzymatic hydrolysis for reprocessing of the waste residue, and biogas production (if co-processed with other wastes). The composition of the algae waste residue was regarded as unsuitable for animal feed/aquaculture, use in the food sector, and use as soil conditioners/compositing, mainly due to certain heavy metals (such as selenium, mercury, iron, copper and boron) and iodine being present at concentrations exceeding maximum allowable limits for these specific beneficial uses (Department of Health, 2018: 114). The relatively low pH (of 4.66) was also a limiting factor for certain of these beneficial uses, and may require neutralisation before further use is considered (Bikker *et al.*, 2020). It is important to note that dilution of the residue will assist in lowering the elements that are out of specification thus making the residue suitable for various applications.

The potential beneficial uses were further ranked using the following criteria: compliance with South African legal framework, moving waste up the waste management hierarchy, supporting the circular economy, economic viability, energy efficiency, compatibility with existing formulations and practical feasibility.

Enzymatic hydrolysis for reprocessing was found to be the most suitable beneficial use within the South African context as well as the most preferred option for the waste generator (Company X), based on its compatibility with existing formulations and its practical feasibility. Enzymatic

hydrolysis of the waste residue offers the potential of reducing production costs, utilising the waste residue for organic matter enrichment in place of procuring organic matter additives.

5.2.3 Conclusions related to RO 3: Determining the optimal conditions of the most preferred identified beneficial use of *Ecklonia maxima* waste residue

The outcome of RO2 determined that enzymatic hydrolysis of *E. maxima* waste residue, for the purposes of reprocessing, was deemed to be the most preferred beneficial use option.

Research objective 3 aimed at determining the optimal conditions for *E. maxima* hydrolysis and reprocessing. In the context of the research, the optimal conditions were considered to be the pre-treatment and enzyme combination, which would yield the highest concentration of total organic matter (TOM) and the highest concentration of total sugars.

Two different pre-treatment options (26% peracetic acid, and 0.1% sulphuric acid) and four different enzyme combinations (Celluclast, Ban, Viscozyme and Viscozyme + Celluclast) were tested for this purpose.

Residues pre-treated with peracetic acid (PAA), generally yielded higher concentrations of TOM and total sugars, than residues treated with sulphuric acid, or those without any pre-treatment. Celluclast and Viscozyme independently produced relatively similar yields of TOM and sugars, which were slightly lower than the TOM and sugar yields of Viscozyme and Celluclast combined. The enzyme combination of Viscozyme and Celluclast yielded the highest concentrations of TOM and total sugars.

Therefore, the optimal conditions, given the variables tested during this research, for enzymatic hydrolysis of *E. maxima* waste residue are pre-treatment with PAA, and enzymatic hydrolysis with Viscozyme + Celluclast.

Enzymatic hydrolysis of the waste residue requires further investigation to optimise the process, great potential lies within the process.

5.3 Recommendations and areas of future research

An in-depth understanding of the composition of the seaweed is critical to understand the options available for the beneficiation and beneficial use of seaweed-related wastes.

For a broader/more specific application of the waste residue the qualifying criteria can be widened by adding additional ranking parameters. Future research could investigate restructuring the qualifying criteria.

Laboratory analysis results are more accurate in comparison to proximate analysis, therefore, existing literature on the composition of the waste residue, should be supplemented by further laboratory analysis (toxicological analysis and detailed structural analysis).

To increase the TOM and sugar yields obtained, detailed optimisation of the enzymatic hydrolysis process should be conducted. Various concentrations of the pre-treatment reagent must be trialed to find one that increases the porousness of the residue.

To increase profitability by reducing costs related to higher enzyme input, a mineral acid or base can be used to solubilise waste residue. The acceptability of this option should, however, be confirmed with regulatory authorities. Pre-treating the residue using a reagent with a lower pH should be further investigated.

Means of processing the algae waste residue (i.e. neutralisation, immobilisation of heavy metals, moisture reduction, etc.) to make the waste residue more suitable for a range of beneficial uses should be explored in future.

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APPENDIX 1

Material Safety Data Sheet

Seaweed Waste Residue

1. IDENTIFICATION OF PRODUCT AND COMPANY

Trade name:	Seaweed waste residue
Chemical name:	<i>Ecklonia maxima</i> (seaweed) extract
Manufacturer:	Company X

2. COMPOSITION AND INFORMATION ON INGREDIENTS

Raw material:	Fresh material from <i>Ecklonia maxima</i>
Additives:	Water and preservative

3. HAZARDS IDENTIFICATION

Possible exposure:	Ingestion, inhalation and skin contact
Eye contact:	Non-irritant
Skin contact:	Non-irritant
Ingestion:	Non-toxic
Inhalation:	Non-toxic

4. FIRST AID MEASURES AND PRECAUTIONS

Eye contact:	Immediately rinse with water for 5 minutes
Skin contact:	Wash skin with soap and water
Ingestion:	Rinse mouth with water and drink a large quantity of water
Inhalation:	Remove product or move person to fresh air

5. FIRE HAZARDS

Flash point:	None to boiling
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Flammable limits:	Not applicable
Extinguishing media:	Use water spray, dry chemical or carbon dioxide
Firefighting procedure:	Remove container from fire area
Combustion products:	Vapours are non-toxic and non-irritant

6. ACCIDENTAL RELEASE MEASUREMENTS

Sweep up waste and place in container for disposal. Wash down area. Uncontaminated spilled material may be reused.

7. HANDLING AND STORAGE

Handling: Avoid eye contact and prolonged skin contact. Respiratory protection is normally not required. Wash hands thoroughly after handling the product.

Storage: Store in the original labelled container in a cool place away from direct sunlight. Keep separate from food and feed products and out of reach of children and animals.

8. EXPOSURE CONTROL / PERSONAL PROTECTION

Personal Protective

Equipment (PPE): Chemical goggles or shielded safety glasses and rubber or neoprene gloves are not required, but their use is recommended.

Respiratory: Protection normally not required, but a respirator can be used

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance: Light brown paste

PH: 4.00 - 4.60

Odour: Faint seaweed

Vapor pressure: Not applicable

Vapor density: Not applicable

Percent volatile: Not applicable

10. STABILITY AND REACTIVITY

Conditions to avoid: Damp or humid conditions

Incompatibility:	Strong bases and oxidizing agents
Hazardous decomposition:	No products known
Acute oral LD₅₀:	>5600 mg/kg in rats – non-toxic
Acute dermal LD₅₀:	>8600 mg/kg in rats – non-toxic
Inhalation LC₅₀:	>5.92 mg/L air in rats – non-toxic
Skin irritation:	Non-irritant in rabbits
Eye irritation:	Non-irritant in rabbits
Chronic effects:	None known

12. DISPOSAL CONSIDERATIONS

Waste disposal: Product should be buried in accordance with any local legislation

Container disposal: Wash thoroughly with water and soap prior to reuse

13. TRANSPORTATION INFORMATION

Not regulated

14. REGULATORY INFORMATION

Classification: This product is considered non-toxic and non-irritant

Date of Issue: 22 July 2010

Latest amendment: 22 September 2021

Important:

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