Biowastes for Plant Production

– A Guide to Plant Biostimulants

Organiskt avfall för växtodling – En Guide till Växtbiostimulanter

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Abstract

Biostimulants are a suggested tool to achieve sustainable plant production. These are products sourced from biological processes or extracted from biological material, which induces physiological responses in plants. Leading to one or several of the following improvements; better nutrient use efficiency, tolerance to abiotic stress, quality traits or increase the availability of confined nutrients in soil or rhizosphere.

This literature review aim to give examples of biological wastes which can be utilized as biostimulants in plant production along with an overview of plant responses. Resulting in a guide which can serve to widen the knowledge about biostimulants and biological wastes which can be relevant for growers, municipalities, industries and horticultural students who wants to explore renewable, circular and biological inputs for plant production. Particularly three biological wastes or by-products are given as examples namely; seaweeds, fish waste and a process called vermicomposting.

Sweden aims to become a leading producer of sustainable “Blue food”, meaning increased economic support for businesses working with fish- and seaweed production, it so happens to be that both of these and their by-products have value as biostimulants. They are even two of the most researched by-products for biostimulants globally, known for their multitude of bioactive compounds that can improve plant growth and quality. Which can lead to increased yields while giving the ability to reduce inputs of mineral fertilizer. The third example, vermicomposting, is rather a process that can generate biostimulants by utilizing numerous different kinds of wastes.

Conclusion is that biostimulants sourced from biowastes is heterogenous and show wide variation in the nutrient composition and the amount and type of bioactive substances. These bioactive substances are most likely responsible for the beneficial effects on plants and can improve nutrient uptake and nutrient bioavailability, increase overall plant fitness and plant’s tolerance to stresses. Because biostimulants are applied in low concentrations they are, by definition, not considered having enough mineral nutrients that is required by plants. In the future, applying a mixed pool of biostimulants may be a way to deliver both the required nutrients and necessary bioactive substances for optimal plant productivity, this will however require more research and analytical tools. For now biostimulants are at least considered safe for humans, animal and environment.

Keywords: Biological Waste, By-products, Organic inputs, Fish protein hydrolysate, Vermicompost, Seaweed extract, Fish waste, Macro algae

Sammanfattning

Växtbiostimulanter föreslås som ett verktyg för att uppnå hållbar växtodling. Dessa produkter är utvunna ur biologiska processer eller organiskt material och framkallar fysiologiska effekter i växter. Detta kan leda till en, eller flera av följande förbättringar; bättre näringseffektivitet, tolerans mot abiotisk stress, kvalitet eller förbättra tillgängligheten av näringsämnen i jord eller i rhizosfär.

Målet i den här litteraturstudien är att ge exempel på organiska avfall som kan nyttjas som växtbiostimulanter inom växtproduktion samt en överblick av aktiva ämnen och växters respons på dessa. Vilket resulterade i en guide som kan öka kunskapen om växtbiostimulanter från organiskt avfall. Detta är aktuellt för odlare, kommuner, industri och hortikultur-studenter som vill utforska

förnyelsebara, cirkulära och organiska medel för växtproduktion. Tre organiska avfall/bi-produkter ges som exempel och dessa är; tång, avfall från fiskeri/fiskproduktion och processen maskkompost.

Sverige strävar efter att bli en ledande producent av hållbar mat från havet vilket betyder ökade ekonomiska stöd för de företag som arbetar med produktion av fisk och tång. Det råkar vara så att dessa två genererar avfall/biprodukter som har värde som växtbiostimulanter. De är t.o.m. två av världens mest efterforskade organiska avfall för växtbiostimulanter idag, kända för deras långa rad av bioaktiva substanser som kan förbättra växters tillväxt och kvalité. Dessa förbättringar kan leda till ökade skördar och samtidigt ge möjligheten till reducerade insatser av mineralgödselmedel. Det tredje exemplet, maskkompost, är snarare en process som kan generera växtbiostimulanter genom användning av flera olika typer av organiskt avfall.

Slutsatserna är att växtbiostimulanter utvunna från dessa organiska avfall är heterogena och visar variation i näringsämnes-innehåll samt sorten och mängden av bioaktiva substanser. Det är dessa bioaktiva substanser som mest troligt ansvarar för de förmånliga effekterna på växter och kan förbättra tillgängligheten av näringsämnen och växters upptag av dessa. Växtbiostimulanter kan också gagna växtproduktion genom förbättrad växthälsa och bättre tolerans mot abiotisk stress. Eftersom växtbiostimulanter appliceras i låga koncentrationer anses de, per definition, inte ha tillräcklig mängd näringsämnen som växter behöver. I framtiden kan en mix av växtbiostimulanter vara framgångsrik för att kunna applicera både näringsbehovet och de nödvändiga bioaktiva substanserna för optimal produktivitet i växtodling. Tills dess är växtbiostimulanter åtminstone säkra för människa, djur och miljö.

Preface

As humanity keeps investigating and developing technologies to unfold the many mysteries of nature, an understanding emerges about the cooperation that inherently occurs in the soil of which plants, and humans, depend upon. By the help of modern technologies humans can start to see things that they couldn't see with their own eyes before, down to gene level. From this progress, an understanding of plants fascinating relationship with other organisms is beginning to unfold in more detail. Where microorganisms such as bacteria and fungi, together with numerous organisms in the soil food web, interact and rely on plants for photosynthesis-products, just like humans do. And that plants in return receive many benefits from the activities of microorganisms. We are what we eat, and what we eat comes from the soil.

I want to give special thanks to Jean W.H. Yong for sharing his bright energy and wise, experienced knowledge in the field of biostimulants, who in the meantime made me laugh many times. Even though he had a busy spring he agreed to be my supervisor for this thesis, which I am very grateful for. And to my dear family and friends I want to give big thanks for cheering me on.

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Abbreviations

FPH	Fish protein hydrolysate
FPR	Fertilizer product registration
PH	Protein hydrolysate
PSII	Photosystem two
Q-PCR	quantitative polymerase chain reaction
RGPR	Root growth promoting rhizobacteria
SOM	Soil organic matter
SWE	Seaweed extract

1. INTRODUCTION

Agri- and horticulture businesses have an urgent and increasing need to produce higher yields for a growing global population, in ways that are sustainable. In 1996, U.N estimated that in 20 years the world's human population would increase to between 7,5 and 8,5 billion, which turned out to be a correct estimation as in mid-2019 the population reached 7,7 billion (United Nations 2019). Modern agricultural practices have since the 1960's been able to produce increasingly greater yields which actually have exceeded the demands of the human population (Waterlow 2000). Much of this success have been due to agrochemical inputs and irrigation which today, are widely used and depended upon. However impressive these technological advancements have been, they have caused detrimental effects on soil, air and water quality (Tilman et.al 2001, Tedengren 2021, Wilson & Tisdell 2001). Production of agrochemicals such as; herbicides, insecticides, fungicides and mineral fertilizers have relied on fossil fuels which are widely known to increase greenhouse gases in the atmosphere attributing to climate change (ibid.). Thus, feeding a growing global population is not the only challenge to plant production. With a changing climate, abiotic stresses on plants are estimated to increase due to periods of drought, waterlogging and extreme temperatures (Tuteja 2012, Dutta et.al. 2020, Vaughan et.al. 2018).

1.1. Limiting Factors in Crop Production

Plants need nutrients provided from a substrate, be it water (hydroponic), field soil or other substrate which also houses the plant roots. Oxygen (O), hydrogen (H) and carbon (C) are most abundant in plants but they are not considered mineral nutrients, because they are obtained from water and air, through the photosynthesis process (Havlin et.al 1999). Mineral nutrients, that plants mainly obtain through the soil medium, can be in organic and inorganic form, being bound to carbon or not, respectively. Mineral nutrients are divided into macro and micronutrients based on the general concentration in plants (Evert & Eichhorn 2013). Of the macronutrients, nitrogen (N), phosphorus (P) and potassium (K), where N and P are considered to be the main limiting factors for crop production because of two things; they are crucial for essential plant physiological processes and they are both fleeting (Vance 2001). These fleeting characteristics are due to nitrogen's volatility in gas form and phosphorus can immobilize quickly, either through being locked up in organic matter by microbes or creating complexes with cations.

Anthropogenic manufacturing of mineral N-fertilizers, has led to depositions of N in environments, which are linked to greenhouse effect, smog, stratospheric ozone depletion, acid deposition, coastal eutrophication and decreased productivity of terrestrial ecosystems and fresh- and marine waters (Galloway et.al. 2004, Møller & Laursen 2015). Much of the Phosphorus used as fertilizer are sourced from phosphate rock, a nonrenewable resource that is becoming scarce and increasingly costly to mobilize, with adverse environmental effects both when mined and applied to agriculture (George et.al 2016). Phosphorus is as mentioned, easily immobilized in soil and thus unavailable for plant uptake, causing accumulation in soils with a continual risk of being lost to waterways where it is culprit for eutrophication.

1.2. Plant Biostimulants Role in Crop Production

In response to the adverse effects of agrochemicals, plant biostimulants are suggested as a sustainable complement. These are a group of substances or microorganisms from natural origin that improves nutrient uptake, health, growth, quality or stress responses in plants (Xu & Geelen 2018, du Jardin 2015, Calvo et.al 2014, Bulgari et.al 2015, Khan et.al 2009). Plant biostimulants may fill in and reduce the inputs of agrochemicals, to achieve successful yields with less damaging effects to terrestrial and marine ecosystems (du Jardin 2015, Rouphael & Colla 2020, Stirk et.al 2020). In other words, these kinds of compounds causes diverse responses in the plants' physiology which activates stages in plant development, growth and can result in protective effects against environmental stresses (Calvo et.al 2014, du Jardin 2015, Canellas et.al 2015, du Jardin et.al 2020).

Examples of plant biostimulants are seaweed extracts, humic and fulvic acids, protein hydrolysates, N-containing compounds, chitosan, and other biopolymers and inorganic compounds (Calvo et.al 2014, du Jardin 2015, du Jardin et.al 2020). Plant biostimulants includes the mentioned substances (non-microbial), and also microbial ones. Microbial plant biostimulants are for example free-living, rhizospheric or endosymbiotic beneficial fungi and bacteria, where mycorrhizal fungi and root growth promoting rhizobacteria (RGPR) are categories (FPR 2019).

1.3. Biowastes As Source of Biostimulants

Adding biostimulants sourced from biological wastes to crop production might be a step on the way of achieving high yields with less pollution (Madende & Hayes 2020). Several bioactive compounds derived from biological wastes has been identified to benefit plant growth in multiple ways, where the most studied products today are algal extracts (seaweed extracts) and protein hydrolysates (Xu & Geelen 2018). The biowastes investigated in this thesis are seaweeds, fish waste and the various biowastes processed through vermicomposting and these three can be manufactured into seaweed extract, fish protein hydrolysate and vermicompost products respectively. This cannot be considered something new, humans have long been adding organic material to soil but with improved ways of analyzing the content in organic inputs researchers are starting to understand how the bioactive compounds affect plants, besides their nutrient content.

1.4. Purpose and Research Questions

Biostimulants are possible tools for making agricultural and horticultural practices non-pollutive, efficient and based on renewable resources for the sake of environmental, social and economic sustainability. Since biostimulants are compounds originating from biological processes and extracted from biological material, biological wastes is a source for biostimulants. Yet, research or reviews performed on biostimulants in Sweden seems limited for example when compared to the knowledge of biological wastes as a source for obtaining bioenergy.

The aim of this thesis is to answer four questions;

1. What biowastes have the potential of becoming widely used as plant biostimulants?
2. What are the active ingredients in those plant biostimulants?
3. What are the required treatments of these biowastes before use and application?
4. What gives most successful yields; usage of one or several biostimulants?

1.5. Limitations

Decision was made to stick with European Union (FPR 2019) definition of a biostimulant, which is mentioned in the first section of the Result section. A limit on the number of biowastes have been set to three and these biological wastes are chosen for their occurrence in Sweden. This literature study does not give facts and figures on the amount of every biological waste mentioned.

There are microbial and non-microbial biostimulants, and herein the former is only mentioned and not discussed in any detail.

2. METHOD

This thesis has been conducted as a literature review where research papers, scientific reviews and reports have been assessed. Utilized through online databases like Primo, Google Scholar and Scopus. Many of the research papers have been found in review papers.

Some of the search words used are;

- biostimulant AND plant AND production
- biostimulant AND waste
- biostimulant AND fish waste / seaweed / vermicompost / humic

3. RESULT

3.1. Background to Plant Biostimulants

A beginning of the term plant biostimulant can be found back in 1951, Filatov then expressed the term “biogenic stimulators” to explain the biochemical restructuring in organisms’ tissue during exposure to unfavorable conditions (Yakhin et.al. 2016, du Jardin 2015). Researchers have phrased other terms along the way such as organic biostimulant, metabolic enhancer, plant strengthener. Thus the original term has gone through a gradual development to recently become; plant biostimulant. A clearer definition of these compounds has been requested by researchers and manufacturers (du Jardin 2015, Yakhin et.al 2017) and in 2019 there was finally an agreed definition in EU. The European Fertilizer Product Registration stated that a plant biostimulant product should aim to improve one or several of the following characteristics of the plant or the plant rhizosphere: (*i)* nutrient use efficiency, *(ii).* tolerance to abiotic stress, *(iii)* quality traits, or (*iv)* availability of confined nutrients in the soil or rhizosphere (FPR 2019).

Biostimulants can be described as complex mixtures of compounds derived from biological processes or extraction of biological materials, which are beneficial to plant productivity regardless of its nutrient content (Yakhin et.al 2017). It is important to underline that plant biostimulants do not affect plants due to their nutrient content but rather on physiological traits of plants, and nutrient efficiency. In fact, in difference to fertilizer products, pesticides and soil amendments, plant biostimulants are applied and effective at very low concentrations (du Jardin 2015, Stirk et.al 2020). Biostimulants may therefore act in addition to fertilizers, making them a tool to optimize nutrient efficiency and thus reducing the total amount of applied chemical/mineral nutrients in agriculture and horticulture (FPR 2019). Applications of plant biostimulants at high concentrations have proven growth inhibitory instead of growth promoting (Finnie & van Staden 1985, Atiyeh et.al 2002, Craigie 2011, Arancon et.al 2008).

3.1.1. Complex Mixtures

The important aspect of biostimulants is that the majority of these products are complex mixtures of compounds, many still unknown (Bulgari et.al 2015). This complexity is considered to be key for biostimulants performance, suggesting that properties of the whole spectrum of compounds possess the beneficial effects on plant productivity (Yakhin et.al 2017). Meaning, organic compounds might not show the same effectiveness as separate parts. Due to this complex mixture within biostimulant products it is not suitable to classify biostimulants based on their composition and researchers have thus suggested; "..*biostimulants should be classified on the basis of their action in the plants or, even better, on the physiological plant responses rather than on their composition*" (Bulgari et.al 2015 pp. 3).

3.1.2. Inputs of Organic Origin - Effects Elicited Besides Mineral Nutrients

Effects from organic input on plant productivity have sometimes proved similar or better compared to a mineral fertilizer (Sultana 2015). This is suggested to attribute to microbe and plant relations. Soil microorganisms are most abundant in the root zone of plants (rhizosphere), where they respond to root exudates released from plant roots (Evert & Eichorn 2013, Frankenberger & Arshad 1995). These released exudates can contain a variety of compounds foremost organic acids and sugars, but as well amino acids, fatty acids, vitamins, hormones and antimicrobial compounds (Turner et.al 2013). Exudates can in turn give microbes energy to degrade organic matter, transform it and release more plant-available nutrients, a process called mineralization. Additionally, microorganisms have the ability to produce plant growth promoting substances. These are for example inactive plant hormones and/or plant hormone-precursors right where the plant needs it; in the rhizosphere (Arshad and Frankenberger 1991, Wong et.al 2016). This quote frames it quite well;

> ”Microbial communities in soil, particularly the rhizosphere, possess great potential to produce a vast range of metabolites (biologically active substances) that may affect plant growth directly after being taken up by the plant, or indirectly by modifying the soil environment. ” (Frankenberger & Arshad 1995, pp 1.)

The plant can synthesize these microbially produced substances into active plant hormones to trigger plant development and other responses (Stirk & van Staden 2010). The term hormone comes from the Greek word *horman*, meaning "to stimulate" (Evert & Eichhorn 2013). It is described that plant hormones distinguish from metabolic processes. Where the latter is providing the building blocks and energy required for plant life and the hormones part is to regulate the growth of individual parts and the speed of growth (Davies 1995 see Gaspar et.al 2003). Plant hormones stimulate physiological responses in plants (Evert & Eichorn 2013) by regulation of plant growth on a cellular level by initiating and continuing cell cycle checkpoints and also, by controlling numerous mechanisms that determines plant physiology, processes of reproduction and responses to stress (Lu et.al 2021, Wong et.al 2016).

Biostimulants are a tool to optimize efficiency of inputs and thus reducing the amount of total applied mineral/chemical fertilizers (FPR 2019). It was understood by Frankenberger & Arshad (1995) that (*i)* organic materials with bacterial presence are more effective than sterilized ones, (*ii*) pure-cultured bacteria show less effectiveness than a mixture of bacteria together with organic material and, (*iii*) organic materials display physiological effects on plants that can't be replaced with an equal amount of mineral nutrients. The last one quite puts the finger on biostimulants role in plant growth enhancement. Namaala & Smith (2020) review on plant growth promoting microorganisms is describing their impact on plants abiotic tolerance. It is recommended for more depth since microbial biostimulants did not fit the scope of this thesis.

3.1.3. The Yield Gap

It is recognized that both chemical and organic fertilizer-regimes have weak links in terms of sustainability. Where extended use of chemical fertilizer deprives the beneficial soil microorganisms, and organic fertilizer are often resulting in lower yields due to unpredictable mineralization of nutrients. Wong et.al. (2015, 2016) envision a "hybrid approach" of both chemical and organic fertilizers, to achieve a better economy, soil health and quality in agri- and horticulture businesses. Similarly, De Saeger et.al (2020) points out the economic disadvantage to organic systems and the environmental disadvantages to chemical ones. Where low-input organic farming yields are estimated to be 5 to 34% less, compared to the high-input chemical systems and using chemical systems solemnly may result in detrimental effects to environment (De Saeger et.al 2020). Biostimulants are suggested to fill this gap.

3.1.4. Plant Biostimulants in Relation to other Amendments

To be clear on the differences and similarities between biological fertilizers, biological control and biostimulants see figure 1. Biostimulants differ from both of these mainly due to (*i*) its active ingredients being effective at low concentrations, and (*ii*) not having enough of the required nutrients to classify as a fertilizer and, (*iii*) does not have direct effect on pests or pathogens (Calvo et.al 2014). Biological fertilizers contain live microorganisms together with nutrients (Macik et.al 2020) and biological control is entirely different, being about living organisms introduced or naturally occurring to control a pest or pathogen (Shields et.al 2019). However, what all of these share is a biotic origin.

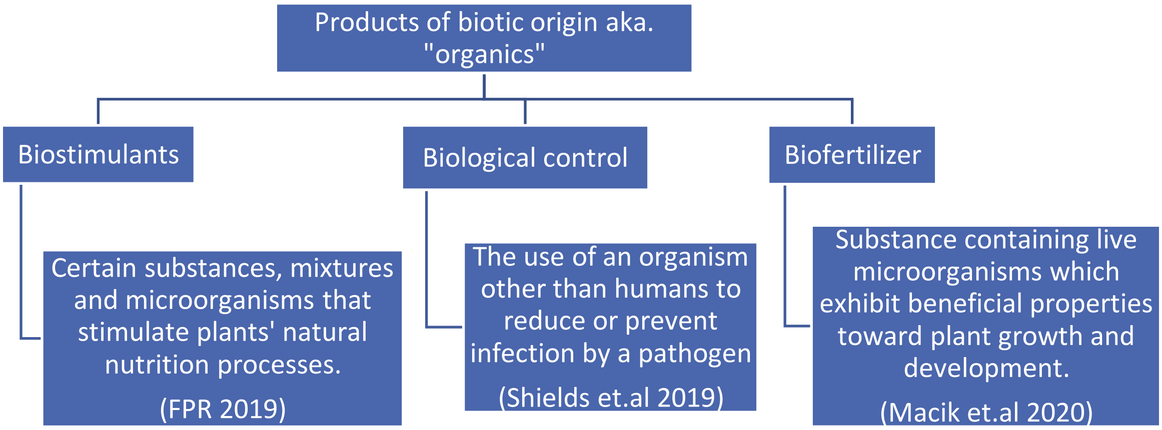

Figure 1. Illustrating similarities and differences between biological tools in plant production.

This first section were meant to give a brief overview on plant biostimulants. Now follows a total of three sections with description of three possible biowastes suggested as biostimulants. Firstly seaweed, followed by fish waste and with the third rather being a description of a process to deal with numerous different kinds of biowastes namely, vermicomposting. Their treatment requirements, bioactive ingredients, plant responses and some of their mineral nutrient content are described.

3.2. Seaweeds

Seaweeds are marine macroalgae which are categorized by their pigmentation, into brown, red and green algae, belonging to the phyla Phaeophyta, Rhodophyta, and Chlorophyta respectively (Evert et.al. 2013). They are plant-like in terms of being a primary producer; they have pigments to perform photosynthesis. There are approximately 10 000 identified seaweeds (Goñi et.al 2020) which have adapted for the last 2.45 billion years (Stengel et.al. 2011). Through challenging environmental conditions such as; shifting temperatures, salinity, nutrient starvation and radiation they have developed a large and diverse arrangement of biochemicals.

Brief History

In human history seaweeds have been used for food, medicine, animal fodder and soil amendments in agriculture. Adding raw seaweed can influence soils both chemically and physically, increase chelating of minerals and affect the soil microbiota in ways that improves soil texture, water holding capacity and general soil health (Calvo et.al 2014, Khan et.al 2009). Since the 1950's raw material from algae have been processed into seaweed extracts (SWE) (Craigie 2011, Al-Juthery et.al 2020), occurring as the leading plant biostimulant on the global market today (Stirk et.al 2020). The mega class Brown algae are most represented in these products because they are common and have impressive rates of growth resulting in high biomass (Sharma et.al. 2014, Yakhin et.al 2017).

The Benefits of Aquaculture

Cultivated seaweed (aquaculture) does not compete with arable land area, and neither acquires inputs of fertilizer, pesticide and fresh water like terrestrial plant production do (Singh et.al. 2011). And algae, cultivated or naturally occurring, also perform ecosystem services such as creating habitats for species of fish and crayfish and nutrient sequestering (Hasselström et.al. 2018, Seghetta 2016). Seghetta et.al. (2016) performed a study on eutrophication reduction and nutrient cycling in Danish seaweed cultivation (*Saccharina latissima*) under different waste management systems. They found that offshore seaweed production had a significant positive impact on eutrophication through bioextraction (nutrient removal), of nutrients such as N and P. Through life cycle assessments of three waste management systems, (i) seaweed as fertilizer, (ii) put to landfill, (iii) incineration with energy production, they concluded that using seaweed as fertilizer had, in terms of marine eutrophication, the lowest environmental impact. Seaweed cultivation can improve water quality by bioextraction of excess anthropogenic emissions and, the seaweed can be recycled as nutrient or biostimulant to achieve a circular nutrient cycling. Where leached and run-off nutrients from agriculture can be retrieved from surface waters and oceans, cycling nutrients back where we need them and at the same time achieve water quality goals (ibid.). In Sweden, seaweed is also suggested for human food (Hasselström et.al. 2020), biofuel production (Wu et.al. 2019) and as a fertilizer (Pechsiri et.al 2016).

3.2.1. Treatment and Application of Seaweed Extracts

Seaweeds have historically been added directly to soil but, have a slow decomposition rate if not chopped to smaller pieces which increases the surface area of the material. During decomposition however, toxic sulfhydryl compounds are produced which can inhibit the growth and seed germination of plants for up to 15 weeks (Milton 1964 see Craigie 2011). It was not practical to transport whole seaweed over long distances and development has thus moved from a compacting method described by Gardissal (1857 see Craigie 2011) to Milton’s liquefied seaweed extract in 1952 (Craigie 2011), to today’s high-technology extraction of bioactive compounds found in seaweeds. The commercial SWE of today are foremost produced from brown seaweeds and the following species, *A. nodosum*, *Laminaria spp*., *Sargassum spp*., *Ecklonia maxima, Turbinaria spp.* and *Durvillaea spp* (Khan et.al 2009, Sangha et.al 2014, Yakhin et.al 2017). As mentioned, brown algae are widespread and generates plenty of biomass, explaining its dominant use.

Manufacturing of Seaweed Extracts

The information about the production of SWE at the moment of writing is limited. This has much to do with manufacturers keeping this information excluded to keep a competitive advantage on the market (El Boukhari et.al 2020). In general the seaweeds are first washed and sometimes also dried before being disrupted. Disruption is made either by cryo-processing or by using high pressure (Stirk et.al 2020), cryo-processing cools or freezes the material and is thereafter grinded/milled (micronization). The processing of seaweeds through cryo-processing enables a mechanical process without organic solvents, acid or alkali, which permits minimal degradation of bioactive substances such as phytohormones and antioxidants (Sharma et.al 2014). The biomass that have been grinded without drying can go through ultrasound treatment or be put through a enzymatic hydrolysis, where the latter can skip an extraction step and proceed straight to a centrifugation and filtration-step (El Boukhari 2020). If not enzymatically hydrolyzed, the extraction of bioactive compounds from seaweeds uses these following methods, either in combination or one method exclusively; (*i*) alkaline, (*ii*) acid and (*iii*) water extraction (Sharma et.al 2014, El Boukhari et.al 2020). A few novel technologies for extracting bioactive substances from seaweeds are mentioned in a review by El Boukhari et.al (2020), which apparently extracts all compounds without affecting their bioactivity. These are; ultrasound-assisted extraction, enzyme-assisted extraction, supercritical fluid extraction, microwave-assisted extraction, and pressurized liquid extraction. As mentioned, details on specific pressure, temperatures, pH and processing times used by industrial manufacturers are not easily obtained (Khan 2009, Craigie et.al 2011). However, alkaline extraction with sodium or potassium solutions with or without heating is the most common practice (Craigie 2011, Stirk et.al 2020). For further depth on the manufacturing processes see review by Sharma et.al (2014) and El Boukhari et.al (2020).

Variables Affecting the End Product

Without surprise, these different methods achieve different content, characteristics and quality of the SWE products. Ranging pH between 4-10, solid content, smell, viscosity, color, shelf-life and the rate of bioactivity are factors that may differ widely between products (Craigie 2011, Sangha et.al 2014, Carrasco-Gil et.al 2018, Stirk et.al 2020). This is of course not only attributed to processing method but also which seaweed species are used, and where and when it is harvested. Geographic location and traits of the site such as water temperature, salinity and exposed or sheltered shoreline affects the raw material and, qualities in the raw material differs between algal families and species (Goñi et.al 2018, 2020). See figure 2 for an overview of variables affecting the composition of SWE. Carrasco-Gil et.al (2018) did a study where four commercial SWE from brown algae were investigated. Three of these were made from *A. nodosum* and one from *Durvillera potatorum*, but all had different extraction processes. These four varied in micronutrient content, pH and in the concentration and type of plant growth regulators. Due to variation in SWE it is suggested by the authors that all commercial products' composition should be analyzed for better speculation and to outline the mechanisms' cause-effect relationships and, of course be able to guarantee the suggested effects to the customer. An additional aspect of complexity is application method and the individual ability of plants to receive or absorb the substances in SWE (Khan et.al 2009).

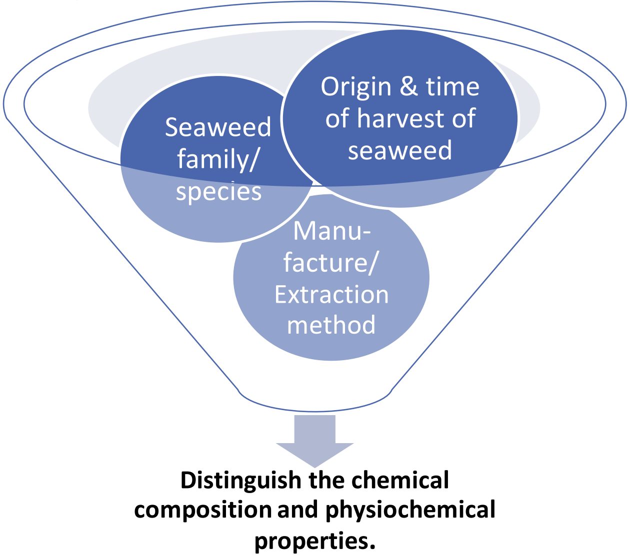

Figure 2. The variables effecting the chemical composition and physiochemical properties of seaweed extracts. (Craigie 2011, Sangha et.al 2014, Carrasco-Gil et.al 2018, Stirk et.al 2020, Goñi et.al 2020).

Application

The final product after extraction can be dried to a powder or prepared into liquid form. Concentration in the latter is diluted, it is thus common to increase the nutritional value. Often done by adding macro- or micronutrients, not seldom chelated trace minerals and such formulations may be customized for specific crops (Craigie 201, Stirk et.al 2020). SWE in liquid form can be foliarly sprayed (Blunden et.al 1996, Kurkani et.al 2019, Colla et.al 2017b), applied as a drench (Elansary et.al 2016), in hydroponic systems (Santaniello et.al 2017) and, in dried powder or pelleted form directly applied to soil and plant roots (Xu & Leskovar 2015). The type of application, concentration, rates and when it is applied influences the plant responses (Sangha et.al 2014, Crouch and van Staden 1992).

3.2.2. Bioactive Ingredients and Plant Responses

The direct effects of SWE's on plants have been reported by many researchers. Being rich in polysaccharides, micro and macronutrients, proteins, poly unsaturated fatty acids, polyphenols, vitamins, osmolytes and plant hormones gives SWE wide-ranging benefits to plant health (Al-Juthery et.al 2020, Gupta et.al. 2011, Michalak et.al 2016, Khan et.al 2009, Ali et.al 2021). However, it has been obscure what the actual mode of action is from this cocktail of organic substances (Khan et.al 2009, Sangha et.al 2014, Craigie et.al 2011). In the following section, some of the suggested mechanisms of the plant stimulatory effects, the contents and the active ingredients will be further explained.

Seaweed Extracts – a Multitude of Organic Compounds

The plant responses to SWE have been reported and summarized by several review papers (Craigie 2001, Khan et.al 2009, Yakhin et.al 2017, Sharma et.al. 2014, Ali et.al 2021, Sangha et.al 2014). They been shown to accelerate plant growth, improve seed germination, nutrient uptake, induce plants tolerance to abiotic stress, amplify crops nutritional quality and improve flowering, fruit development and yield. Finnie & van Staden (1985) showed by ashing a concentrate of *Ecklonia maxima* that in fact, the organic fraction hosts the plant growth effects. Since the earlier, significant results on improved root and shoot growth of tomato roots (in vitro) did not persist after ashing. Also, when fractions of SWE have been applied these have not been able to recreate all the effects seen from whole SWE applications. Indicating a synergy effect of several active organic components hosting the plant growth responses of SWE (Ali et.al 2021, Billard et.al 2014). The mechanism of SWE are generally complex, much because of this multifold of components, where one component might act on several different of the plants metabolic networks (Pohl et.al 2019), and due to that two or more genes (polygenic) can be responsible for responses "*implicated in such intricate and dynamic processes..*" (De Saeger et.al 2020:595). In studies of abiotic stress, the SWE application have sometimes only shown beneficial effects under stress and not under optimal conditions (Xu & Leskovar 2015). This complexity can explain why it is challenging to elucidate SWE mode of actions but much is indicating that SWE treatment impacts genetic pathways, thus influencing several molecular and biochemical changes (Khan et.al 2009, Sangha et.al 2014, Ali et.al 2021).

The SWE are estimated to consists of 60,92% carbohydrates, 15,43% protein, lipids less than 3%, minerals less than 2% and plant growth regulators less than 2% (see figure 3). Plant growth regulators is just another word for plant hormones which in addition can be described as plant growth hormones or plant growth substances. Moreover, hormones can be plant growth promotors or inhibitors depending on what processes they are involved in. For more detail on what bioactive substances have been reported in SWE from all three mega classes, see table 7 in the appendix where they are listed by category.

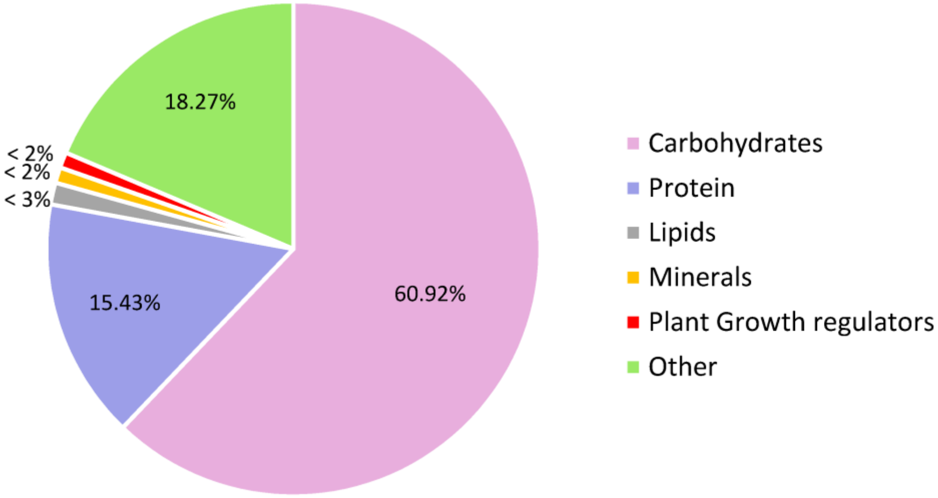

Figure 3. The estimated composition of seaweed extracts belonging to the three mega classes of seaweeds (red, green and brown). (Ali et.al 2021)

The Role of Biostimulants in Improving Plants Nutrient Content

Crouch et.al (1990) proved that a SWE increased the yield and nutritional quality of lettuce, however, this significant increase occurred only in plants that received a double nutrient solution and not in the other two treatments under a lower nutrient dose. The concentrations of calcium, magnesium and potassium was significantly increased in the lettuce but interestingly, root size couldn't account for any extra nutrient uptake because in this case, root to shoot ratio didn't increase for neither of the treatments.

Another study investigating improvement of nutritional value (biofortification) of Winter oilseed rape, *Brassica napus* (Billard et.al 2014). They tested two biostimulants; one being SWE and the other one, humic acid extracted from black peat. The control and treatments received the same amount of liquid fertilizer and any minerals present in biostimulants were considered negligible. Results indicated that both of these stimulated plant growth and nutrient uptake or translocation of nutrients, which took its form in the plants as improved root biomass and increase of chloroplasts per leaf cell. The concentration of following minerals; sodium, manganese, copper, and magnesium were increased compared to control while nutrients such as silicon, phosphorus, nitrogen, calcium, and boron did not have significantly higher concentration compared to control. However, due to the biomass increase from the two biostimulant-treatments, the content of those nutrients was of course higher, and this followed a pattern similar to the growth by an order of magnitude. Overall conclusion drawn from this study was that these extracts have potential of increasing the nutrient content of sulphur, iron, zinc, manganese, and copper in *B. napus.*

Biostimulants being applied in low concentrations may also translate that if there are any mineral nutrients in the biostimulants they are most likely not sufficient to support the mineral nutritional needs required for optimal growth of plants. But it might stimulate plant growth so that the need of mineral fertilizers can be reduced. A study on tomato plants applied with a biostimulant showed just that. With reduced nitrogen-phosphorus-potassium (NPK) fertilization, quality was intact and fruit yield similar to control, while also oxidative stress in leaves were prevented (Koleška et.al 2017). This was not a biostimulant from seaweed but a mixture of polysaccharides, proteins, polypeptides, amino acids, humic acids, hormone precursors and vitamin complexes.

Plant Hormones

SWE effects on physiological features of plants are recognized to be caused by plant hormones (Stirk et.al 2020), but these effects have been referred to as hormone-like activities for many years (du Jardin et.al 2020). Specifically, SWE made from brown algae *A. nodosum,* are concluded to support plants' endogenous balance of plant hormones (De Saeger et.al 2020). Plant hormones serve many important processes in plants, from regulation of physiological growth to responses towards environmental conditions (Evert & Eichhorn 2011). This regulation of the hormonal balance will have effect on (*i*) the plants' homeostasis, a condition of optimal function within cells, (*ii*) regulation of transcription of relevant transporters for nutrient uptake and assimilation, (*iii*) protect and stimulate photosynthesis apparatus and, (*iv*) lowering of stress-induced responses.

In the case of SWE, physiological processes in plants are mainly elicited by auxin, cytokinin, gibberellin, abscisic acid, ethylene, brassinosteroids, jasmonates (Khan et.la 2009, Pohl et.al 2019, Stirk et.al 2020). Plant hormones occur naturally in plants at very low concentrations, and as well in SWE. The amount in seaweed dry weight can be less than 25 nanograms g1− and in SWE even less, pmol ml−1 (Stirk et.al 2020). It is suggested that, due to plant hormones low concentration in SWE, their main mode of action are activation of biosynthetic pathways and hormonal crosstalk network. This last-mentioned, hormonal crosstalk network, is a term for this complex network of synergistic, antagonistic and additive interactions between the different hormones (Aerts et.al 2021, Wong et.al 2016).

Cytokinin Has a Crucial Role

It is commonly accepted that cytokinin is important in crop production because of its relation to increased crop productivity (Li et.al 2016) and many of the SWE induced beneficial effects on stress tolerance can be related to cytokinin activity (Khan et.al 2009). Jameson & Song's (2015) review of cytokinin research so far, summarizes that this plant hormone elicit cell-division and differentiation of cells, shoot and root growth, delay of senescence, transduction of nutritional signals, fruit and seed development, apical dominance and lastly, responses to biotic and abiotic stressors. During early stages of seed and fruit development, levels of cytokinin are elevated and those levels concur with nuclear and cell divisions, thus determining the final seed size (Jameson & Song 2015). Because of this, cytokinin is considered to be a limiting factor to yields. Cytokinin's part in cell division is demonstrated in figure 4, where the related hormonal crosstalk also is included.

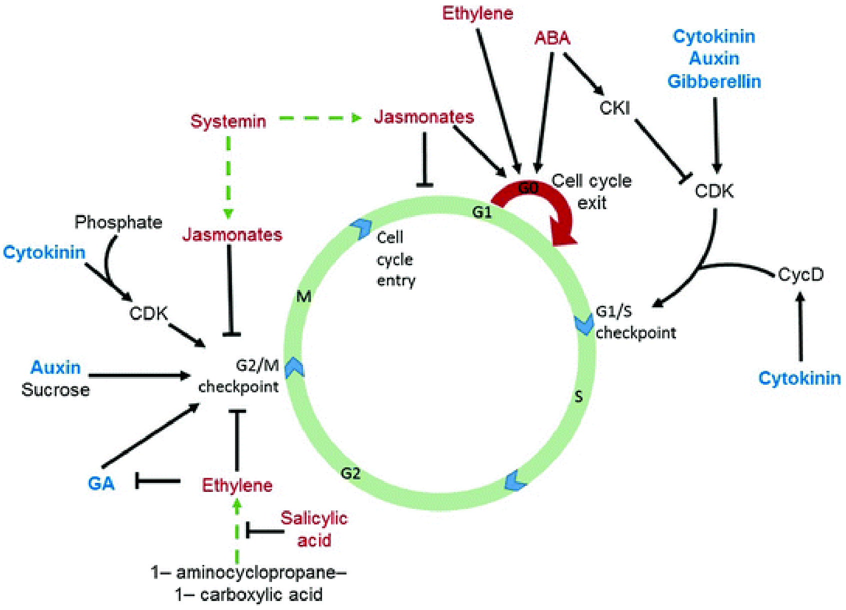

Figure 4. Illustration of cell cycle checkpoints and related hormonal crosstalk network. Used with permission from Yong, J.W.H (Wong et.al 2016).

Environmental Stress

In stress responses, cytokinin interacts with ethylene, auxin and abscisic acid (ABA). The latter is for example, synthesized in plants when levels of cytokinin is high (Stirk et.al 2020). And such a reduction of cytokinin under drought stress show reduced shoot growth but enhanced root growth, as to prioritize a deeper root system where water might be found (Li et.al 2016). Moreover, this reduction of cytokinin levels, and increase of ABA leads to following reactions; stomatal closing, biosynthesis of anthocyanin enhancement which is an antioxidant system, and cell membrane integrity along with accumulation of osmoprotectants. The last-mentioned can be called guards of cells, they are neutrally charged, small, organic molecules that protects the cell from osmotic stress. All of these mechanisms, where cytokinin clearly plays a role, results in an increased tolerance to drought by reduction of cellular dehydration and less oxidative stress (Stirk et.al 2020, Li et.al 2016). The interaction mentioned between high levels of cytokinin and ABA synthesis is one example of a crosstalk network, and cytokinin's interaction with ABA, ethylene and auxin to induce stress responses is another (Stirk et.al 2020). This complex communications network of hormones is responsible for plants' ability to successfully cope with abiotic and biotic stressors (Aerts et.al 2021).

Additionally on the topic of drought stress, Blunden et.al (1996) found an increase in drought tolerance for multiple cultivars when foliarly sprayed with alkaline *A. nodosum* SWE. When applied to the soil, all species had higher concentrations of chlorophyll in the leaves compared to control that received equivalent amount of water.

Moreover, another study investigated hydroponic-grown *Arabidopsis thaliana* (the thale cress) tolerance to drought stress, where plants had been pretreated 5 days before drought with an acidic-produced *A.nodosum* extract (Santaniello et.al 2017). Results showed that treated plants, already prior to the 5 day drought stress, had reduced their stomatal conductance and transpiration rate compared to untreated plants, significantly, with 55% and 53% respectively. On the third day of drought the damages to photosynthetic apparatus on the untreated plants were detrimental with a 90% death rate. While the treated plants instead were able to maintain a 90% relative water content and, keep the potential photochemistry of Photosystem II through to the end of the 4-day dehydration period. Also, by performing quantitative polymerase chain reaction (qPCR), a laboratory technique of molecular biology based on the polymerase chain reaction, they could in real time monitor any increase of certain targeted DNA (marker genes). This made it possible to detect the expression of marker genes connected to any of the processes. Results from qPCR showed that the pretreatment of SWE reduced expression of two marker genes, one involved in ABA-synthesis, and the other stomata regulation. Additionally, increased expression of two other marker genes, being ABA-responsive genes, were noted. Furthermore, towards the end of the drought period significant differences occurred on marker genes related to photoprotection mechanisms of Photosystem II (PSII), such as antioxidant defenses preventing oxidative damage. The amount of RNA, which may activate several genes, were higher in SWE-pretreated plants than in untreated plants which might explain the more intact PSII in the treated plants. For example, an expression of a gene involved in the late stages of antioxidant anthocyanins-biosynthesis (Dihydroflavonol reductase) was higher already at the end of pre-treatment period, but also during the drought period. This was suggested as one reason for the improved defense towards oxidative stress to PSII. The exact mechanism behind this improved drought tolerance from SWE is difficult to track down due to the complexity and variety of metabolites in SWE (De Saeger et.al 2020) which also is true for mechanisms involved in plant growth (Stirk et.al 2020, Ali et.al 2021).

In seed germination of chili, Dutta et.al (2019), showed significant increase in germination percentage, vigor index and seedling weight along with a decrease in the mean germination time compared to control when primed with a liquid from two brown seaweeds, *K. alvarezii* and *G. edulis.* Reasons for these increases were referred to presence of plant growth regulators such as gibberellin, carbohydrates, vitamins, cytokinin, abscisic acid and also possibly the mineral nutrients. Additionally, the SWE primed seed’s antioxidant content were found to be significantly higher than in the other two treatments primed with water only and no primer.

Brassinosteroids

This group of plant hormones act in the whole plant and are important in many stages of a plants life, both promoting general plant growth and stress tolerance. They can influence cell elongation, seed yields and development of fruit and flowers (Stirk et.al 2020, Nolan et.al 2020). In terms of environmental stress brassinosteroids stimulates production of reactive oxygen species (ROS) and proline which results in mediation of osmotic pressure, maintaining stability of membranes, cause activation of stress-related genes and create reactive oxygen species (ROS) scavengers (Stirk et.al 2020). These ROS scavengers are enzymes, also called antioxidants because they neutralize potentially damaging oxygen molecules. Moreover, this hormone function as signals to genes that for example are involved in cell division and elongation, bending, development of reproductive organs and vascular development (xylem and phloem) (Wong et.al 2020).

Carbohydrates

There are unique carbohydrates found in seaweeds that are not found in terrestrial plants and, as seen in figure 3, they make up about 60% of the organic fraction in SWE. These are classified into agars, carrageenans, alginates, fucans and phlorotannins (Stirk et.al 2020). Other carbohydrates found in seaweeds are ulvan, laminarin, cellulose/hemicellulose. There are several parameters that defines a carbohydrate, such as structural characteristics and molecular mass. Depending on molecular mass carbohydrates are categorized as; poly-, oligo-, di- or monosaccharides and depending on structural characteristics they are e.g. assigned as a homopoly- or heteropolysaccharide (Goñi et.al 2020). These parameters also determines bioactivity, and because there is such diverseness amongst seaweed carbohydrates their composition is challenging to analyze and even more so, hard to associate their mode of action (Goñi et.al 2020). However, carbohydrates are so far suggested as part of the bioactive ingredients in SWE which may enhance growth and plants’ defense system. This is referred to auxin-like responses on root growth and their involvement in the signaling network related to successful abiotic and biotic tolerance (Bulgari et.al 2015, Sangha et.al 2010, Goñi et.al 2020). For example, carbohydrates can act as signaling molecules similar to hormones that induces immunity responses in plants. This immunity response from sugars/carbohydrates have given birth to the term; sugar-enhanced defense or high-sugar resistance (Goñi et.al 2020). The main roles of sugar in a plant that is under attack from e.g. a fungi can be as structural material to rebuild or strengthen broken cells, as signal molecule in the hormonal signaling network, and thirdly providing energy for this defense response (Morkunas & Ratajczak 2014).

Betaines

This is a group of active compounds in SWE. They can work as osmoprotectants in plants, reducing damages on cells from drought, salinity stress and other oxidative stress (Pohl et.al 2019, Khan et.al 2009). Particularly, glycine betaine has supported survival and better growth in many cultivars under stress conditions (Colla et.al 2015). Additionally, betaines have been regarded to keep chlorophyll-levels intact or improved, likely by decreasing the degradation of chlorophyll (Khan et.al 2009, Blunden et.al 1996).

3.2.3. Mineral Nutrient Content in Seaweed Extracts

Even though biostimulants by definition show effects on plants regardless of nutrient content (Yakhin et.al 2017) the nutrient effect cannot always be negligible (Xu & Mou 2017). Michalak et.al (2015) performed an extraction technique, supercritical fluid extraction, on Baltic seaweeds harvested in Poland waters and the species were *Ulva*, *Polysiphonia* and *Cladophora*. See table 2 for a listing of mineral nutrient content of the seaweed biomass and table 3 for a comparison to see mineral nutrient content of the extract.

Table 1. Listing of mineral nutrient content in Baltic seaweed biomass (Adapted from Michalak et.al 2015).

Macronutrient	**N**	**P** **g kg -^{1}** **DM**	**K** **g kg -^{1}** **DM**	**Ca** **g kg -^{1}** **DM**	**Mg** **Mg kg -^{1}** **DM**
Baltic Seaweeds	-	1,52	4,93	14,4	4,07

Table 2. A seaweed extract made from Baltic seaweeds, produced by superfluid extraction technique. Mean value and standard deviation (S.D.) shown in Milligram per Litre (Adapted from Michalak et.al 2015).

Macronutrient	**N**	**P** **Mg L-^{1}**	**K** **Mg L-^{1}**	**Ca** **Mg L-^{1}**	**Mg** **Mg L-^{1}**
Seaweed Extract Mean value and (S.D.)	-	43 (6)	52 (8)	1060 (210)	406 (61)

That concludes the seaweed section where history, treatment requirements for SWE and some of its bioactive ingredients have been described. For a more comprehensive look on known bioactive compounds in SWE along with other aspects on Biostimulants see a recent book, *The Chemical Biology of Plant Biostimulants* edited by Geelen and Xu (2020).

3.3. Fish Waste

As a response to the environmental challenges facing the world, Europe is aiming for a functioning bioeconomy (European commission 2017). Promoting a circular economy, aiming to minimize wastes and pollution when sustainably producing renewable resources. Fisheries and aquaculture are mentioned as part of that circular economy, being a source for renewable food, fibre, feed, bio-based products and bio-energy. These resources are clearly meant to suit different uses, as there is a lot of research for fish wastes used as food additives and for their pharmaceutical properties (Gao et.al 2021, Chalamaiah et.al 2012, Halim et.al 2016).

The Fish Waste

At present, the amount of harvested food from the sea is 59 mega tons (Mt) globally, and Costello et.al (2020) suggests that food from the sea can increase to 80-103 Mt in 2050. This will also mean an increase of by-products, since the live weight at present are 102 Mt, and in 2050 possibly around 159-227 Mt. That would make out 79-124 Mt of possible fish waste destined for by-products, if the usage of fish for consumption remains the same as today standards. The post-harvest losses from fish industry are a major concern occurring in most of the distribution chains globally. FAO (2018) estimate it at 27 percent and similarly, during fish processing large amounts of solid and liquid wastes are generated (Arvanitoyannis & Tserkezou 2014). Fish waste or, as it could be called in a circular economy; by-products from industrial processing may still contain 70% fish and shellfish (Olsen et.al. 2014). This content, and other animal derived protein hydrolysates, are rich in amino acids, peptides, fats, mineral elements and other organic compounds (Colla et.al. 2015). Norway, a neighboring country to Sweden with a large fishing industry, did in 2011 dump as much as about 200 000 tons of by-products into the ocean after off-shore processing (Ramirez 2013 See Olsen et.al 2014). In contrast, the by-products from Norwegian farmed fish industry were better utilized. Suggesting that a better solution to deal with by-products off-shore is needed to succeed with a circular economy.

Sweden has increased both its export and import of fish and fish products, sitting amongst the top ten in both categories between 2006-2016 (FAO 2018). In addition, the Swedish Research Council, Formas (2021) did in 2020 make the largest funding in the Swedish aquaculture history. With 48 million in Swedish currency, aimed to promote the development of sustainable aquaculture by circulating resources to agriculture and plant production. Within these projects also the fish manure from aquaponic systems are investigated as a resource to benefit crop production (SLU 2020).

3.3.1. Treatment and Application

The protein hydrolyzates (PH) are sourced from raw material with either plant or animal origin (Cavani et.al. 2006, Ertani et.al 2009, Colla et al. 2015). Other animal derived PHs are from other food production such as leather by-products, chicken feathers and blood meal, and the plant-derived PH's have commonly been from alfalfa hay, legume seeds and vegetable by-products (Colla et.al 2015). The focus in this chapter lies on fish by-products in particular. There has been reported processing of fish by-products on common species in Scandinavia see table 4. The studies referred to in this table are all with intention to use the FPH for pharmaceutical or food-additive purposes.

Table 3. Listing of the type of body part, from which fish and ways of treatment with the recovery of protein (%) and in some cases also nitrogen (%).

Type of fish and part(s) of fish	***Treatment***	***Source***
Salmon. Head	Enzymatically hydrolyzed using Alcalase at optimum substrate to enzyme ratio, temperature and pH to produce hydrolysates. Protein recovery was between 47% and 70%.	Gbogouri (2006)
Herring. Head, whole fish, body and gonad	Enzymatic hydrolysis using Alcalase as the hydrolyzing enzyme with incubation for 75 minutes. Note that all of the parts were hydrolysed both together as whole herring, and as separate parts then made into a powder. All of the FPH powders had desirable essential amino acid profiles and mineral contents (as food additive). Freeze-dried FPH powder contained between 77% and 87% protein.	Sathivel et al. (2003)
Atlantic cod and Atlantic salmon. Fish frames without heads	Enzymatic hydrolysis where the industrial enzymes (Neutrase and Alcalase) and pepsin were used, which were tested with different times and temperature for hydrolysis. 120 minute hydrolysis showed significantly highest protein recovery; cod treated with pepsin and salmon treated with Alcalase had 64% and 67.6% respectively.	Liaset et al. (2000)
Atlantic salmon. Muscle proteins	Hydrolyzed enzymatically with four different alkaline proteases (enzymes that breaks proteins). Protein recovery ranged between 71.7 to 88.4%. Nitrogen recovery was between 40.6% and 79.9%.	Kristinsson & Rasco (2000)

The production of fish protein hydrolysate (FPH), starts with solubilizing the by-products from fish processing with water (Madende & Hayes 2020). This is performed in tanks typically in a ratio of 1:1. Hydrolysis is any chemical reaction in which a molecule of water breaks one or more chemical bonds. There are mainly two ways to perform the hydrolysis, chemically or enzymatically however, the general scope of the process is that larger proteins are being broken down into smaller chains of soluble peptides which contain between 2-20 amino acids (Madende & Hayes 2020, Colla et.al 2014, 2017a). When the desirable rate of hydrolysis is reached the process is stopped by chemical or thermal treatment (Petrova 2018). Now the FPH can be heated as to reduce any microbial activity and fish oil may be filtered out to steer clear of any fat oxidation to occur in the final product (Madende & Hayes 2020). Afterwards it might be necessary to decrease the water content by concentrating the protein mixture where additional fractionation might be wanted to further concentrate the fish proteins, through micro-, ultra- or nano-filtration. This can make an approaching drying treatment more efficient however, demanding additional equipment as well as energy (ibid.). The FPH can come in liquid or dry form and determines the storability, transport and application requirements. The dried FPH is normally stored at 4 °C as a soluble powder or in granulate form (Madende & Hayes 2020, Colla et.al. 2015).

Chemical hydrolysis

The market in horticulture for protein hydrolysates had in 2015, more than 90% of its products based on protein with animal-origin produced through chemical hydrolysis (Colla et.al. 2015). All the peptide bonds of proteins are broken apart by this procedure resulting in a high total content of free amino acids. However, several amino acids and vitamins are destroyed together with a conversion of free amino acids into D-form instead of L-form. This transformation might make PH a worse candidate to plant health because proteins in living organisms have amino acids in the L-form, making the amino acids in D-form unusable to plant metabolism (Cavani et.al. 2003). In chemical hydrolysis two processes are available, acid or alkaline and either of these bring about higher salinity of the PH (He et.al. 2013, Colla et.al 2017a). Acid hydrolysis enquires a high temperature of more than 121°C with pressures over 220.6 kPa. Most common acids to perform the hydrolysis with are hydrochloric acid, but also sulphuric acid can be used. The alkaline hydrolysis is a more simple process where heat makes the proteins break apart and then an alkaline agent is added (Ca, Na or K hydroxide) with the temperature kept at a set point (Pasupuleti and Braun 2010 see Colla et.al. 2015).

Enzymatic hydrolysis

The enzymatically produced PH are more recently introduced and therefore less common and mainly used for the production of PH from plant origin (Colla et.al. 2015). The enzymes used to hydrolyze proteins can be from various sources such as animal organs, microorganisms and plants. Because these are different enzymes they are specific in which peptide-bonds they target. The PH product can therefore be a mixture of amino acids and peptides with diverse lengths, and also lower salinity in comparison to chemical hydrolysis (ibid.). Also, an enzymatic hydrolysis doesn’t need high temperatures but a steady temperature (below 60 ˚C) and steady pH (Colla et.al 2017a). It is proposed as a more environmentally friendly production with lower energy demand and carbon dioxide emissions than the chemical hydrolysis (Bernabei 2015 see Colla et.al 2015). When PH from fish is produced for food-additives or nutraceuticals, enzymatic hydrolysis is preferred to achieve higher quality and more bioactive and bioavailable hydrolysates (Chalamaiah et.al. 2012). Araujo et.al. (2021) noted a 79% reduction in the fish waste going to landfill, where enzymatic hydrolysis resulted in three separate products; collagen, oil and protein hydrolysate.

Depending on the form of hydrolyzation and the given raw material, the final PH product can vary largely at both peptide concentration and molecular weight distribution. The latter can range between several hundred to several thousand Daltons, where Quartieri et al. (2002 see Colla et.al. 2017a) observed that peptides having low molecular weight had the highest plant biostimulant action. Moreover, Colla et.al. (2014) comments on adverse effects such as; plant toxicity and growth depression, recorded from animal-derived PH in comparison to plant-derived. Stating that the higher content of free and, particularly, small amino acids and higher salinity seem to be the culprit. To identify biostimulants in PH a few methods can be mentioned. Eco-toxicological tests, amino acid analysis and gel electrophoresis with dodecyl sulphate polyacrylamide (Madende & Hayes 2020). For even further depth, and review on some treatment methods not mentioned in this text, see Ahuja et.al (2020).

Application

Protein hydrolysates can be applied as seed treatment, to the roots of plants (soil drench) and as foliar spray (Colla et.al 2015, Halpern et.al. 2015). Kolomazník et.al. (2012), on the topic of foliar application, suggests that diffusion controls the uptake of biostimulants and physio-chemical properties, such as lipophilicity and molecular size of the applied medium influences diffusion. Protein molecules probably penetrate the leaf cuticle, which is mostly of lipid material, through leaf stomata and pores. The biostimulant need to be soluble in water and to achieve a successful foliar application, it is necessary that the biostimulant remains in liquid phase long enough on the foliage. Therefore, after rainfall is a good time to spray field grown crops and in greenhouse conditions, the relative humidity should be near the saturated phase (ibid.).

Application of PH will result in competition for the peptides and amino acids with microorganisms in phyllo- or rhizosphere however, there is still indirect effects gaining the plant because of the inherent relations between plant and microorganisms. Luziatelli et.al (2016) confirmed the change on phyllospheric microbial community on lettuce plants with foliar application of two plant based PH. Several beneficial taxa were found related to phosphorus solubilization and indole acetic acid (IAA) production. Lettuce growth was enhanced along with increased leaf chlorophyll and, on top of that, all of the isolated *Bacillus* strains revealed an inhibitory activity against plant pathogens. Another study performed on Lettuce investigated the synergistic effects of foliarly applied, microbial-based biostimulant along with a PH (plant-derived) (Rouphael et.al 2017). This combination resulted in higher total root length and surface, improved chlorophyll synthesis and greater accumulation of the amino acid proline. Exogenous application of proline can improve tolerance to salt stress through regulation of the endogenous proline metabolism. This increased marketable yield with 46,7% compared to untreated plants and 15,5% for the microbial-only treated plants.

These results implies, together with Wong et.al (2016), that biostimulants have direct and indirect effects on plant health. The added biostimulatory substances can act either by direct plant uptake or indirectly, by microbes mediating the benefits through e.g. enzymatic hydrolysis of the peptides/amino acids or the production of phytohormones and/or precursors (Colla et.al 2015, Wong et.al 2016).

3.3.1. Bioactive Ingredients and Plant Responses

Fish protein hydrolysate (FPH) have been examined in relation to plant production in numerous studies with results indicating beneficial plant responses. Lettuce (*Lactuca sativa*) applied with a commercial FPH three times with 300 mL at 0, 14 and 24 days after transplant showed enhanced overall growth (Xu & Mou 2017). The treatment significantly increased chlorophyll content, leaf number from 22 to 28 leaves, fresh and dry shoot weight, as well as root weight. Xu & Mou (2017) could not confidently exclude the nutrient effect from the PH but to explain the growth improvement in their study, the authors referred to suggestions from other researchers. Increased soil microbial activity might attribute enhanced micronutrient solubility and mobility, altering plant root architecture where root length, density and number of lateral roots are amplified and as well, increase of soil enzyme activities that supports nutrient metabolism (Colla et al. 2014, 2015; García-Martínez et al., 2010; Lucini et al., 2015 See Xu & Mou 2017).

Greenhouse grown grape tomatoes, with organic fertilization and a sub-irrigation method, with the addition of 120% FPH gave on average the best response on biomass production compared to control (García-Santiago et.al. 2021). Therefore suggesting that gaps in yield, between organic and conventional production of grape tomatoes may decrease with FPH.

An earlier study on papaya (*Carica papaya L.)* looking into frequency and interval differences of two treatments; Acetylthioproline (AP), a cyclic sulfuric amino acid with similar structure to that of proline (0 and 0.25 $g \cdot L^{-1}$) and a commercial complex of peptides and free amino acids (APC) (0 and 3.0 g·L-1). Results proved that a more frequent application of the biostimulants, with a monthly interval starting first day after flowering and 180th day being the last, gave fruit yield increases at 18% for AP and 22% for APC (Morales-Payan & Stall 2003). Their study didn't give proof of the physiological mechanisms behind yield enhancement but, they noted former researchers' associations between amino acids and peptides to changes in plant secondary metabolism and enzymatic processes, in particular oxidation/reduction systems. Secondary metabolism is the production of compounds which can enable the plant to respond to environmental cues.

Individual amino acids

The free amino acids in PH are ready for uptake directly and can aid the plant in its synthesis of amino acids, making reconstruction quicker and saving energy which might be of importance under abiotic or biotic stress (Madende & Hayes 2020). Also, amino acids can be used as a source of nitrogen (Halpern et.al 2015). Another function of free amino acids are as chelators of metal ions, making the metal a neutral complex rather than a charged ion. Thus the metals are more bioavailable to plants, aiding absorption and transportation of metals from the soil. Which can explain increased mobility and solubility of micronutrients such as Fe, Mn, Cu and Zn. Madende & Hayes (2020) further notes that amino acids such as asparagine, glutamine and cysteine are important for the chelation of Zn, Cu, Ni, Cd and As, in addition decreasing plant toxicity by these heavy metals.

Abiotic stress

Under abiotic stresses such as salinity, high temperature and drought, amino acids (glutamine, proline and alanine) can stabilize proteins, membranes and enzymes. Leading to protection of plant cell structure and function thus continuing the plants water uptake and retention during these kind of stressors (Madende & Hayes 2020, Jiménes-Arias et.al 2021). These amino acids are in that case called osmoprotectants, because they adjust the osmotic pressure avoiding denaturing of essential plant molecules. Qualities in biostimulants as osmoprotectants are explained in recent literature by Jiménes-Arias et.al (2021).

3.3.2. Mineral Nutrient Content in Fish Protein Hydrolysate

Depending on what part of the fish that is treated by hydrolysis, the composition of nutrients will vary between species, depending on size of fish and what tissues are either excluded or included (Ahuja et.al 2020). Where scales and bones, for example have rather high P and Ca content, and scales in particular have high N. For marine capture and inland capture the N-P-K average values have been found to be 130:16:11 and 120:11:13 respectively (Bogard et.al 2015 see Ahuja et.al 2020). Table 5 shows the N, P, K, Ca and Mg content of fish bone hydrolysate from different species. For further depth I recommend Ahuja et.al (2020) who summarizes the nutritional composition recorded from several studies.

Table 4. Mean value and standard deviation (S.D.) of the mineral nutrient composition of 9 different fish wastes in gram per kilo of amount dry matter (DM). From fish bones in particular where 1 from fish heads and 8 from bones (Adapted from Ahuja et.al 2020).

	Macronutrient	**N**	**P**	**K**	**Ca**	**Mg**
Type of fish waste	***Type of fish***	% DM	g/kg DM	g/kg DM	g/kg DM	g/kg DM
Hydrolysed bones from heads of						
	Cod	5,04%	120	1,7	300	3,8
Fish bones from various fish						
	Cod	6,21%	123	0,006	206	3,3
	Blue Whiting	7,15%	93	0,03	181	3,4
	Salmon	4,92%	85	0,009	142	2,3
	Trout	5,31%	92	0,008	155	2,5
	Herring Small	6,43%	101	0,005	173	2,8
	Herring Large	5,02%	99	0,008	205	3
	Mackerel	4,37%	90	0,007	150	2,7
	Horse Mackarel	4,44%	114	0,005	239	3,7
	Mean and (S.D.) Ca (n = 8)	**5,50%**	**100,00 (13)**	**0,006 (0,002)**	**182,00 (34)**	**3,00 (0,5)**

This concludes the section on fish waste and its biostimulant product; fish protein hydrolysate.

3.4. Vermicompost and its Leachate

> “Vermicomposting is a self-promoted, self-regulated, self-improving, self-driven, self-powered and self-enhanced, low or no energy requiring zero-waste technology, easy to construct, operate and maintain.” (Shania et.al 2010:880).

The product *vermicompost* or *vermicast*, and its process *vermicomposting* are widely used globally to produce worms, as a way to deal with organic wastes and increase biosafety of wastes (Sherman 2018, Swati & Hait 2018, Eastman et.al 2001, Sinha et.al 2010). Composting is a decomposition process of organic material and vermi, is the latin word for worm. Vermicompost is the product of vermicomposting; a decomposition process using earthworms. In other words written by Sherman *“Vermicomposting is a process that relies on earthworms, and microorganisms to break down organic matter and transform its biological, physical and chemical characteristics into a stable product..”* (2018:8)

3.4.1. Earthworms in Vermicomposting

Earthworms have been divided into three main groups, epigeic, anecic and endogeic, based on their habitat and behavior (Wong et.al 2020). The epigeic earthworms and in particular the species *Eisenida fetida* and *Eisenida andrei*, are the most commonly used for large scale vermicomposting (Dominguez & Edwards 2010). The reason being their short life-cycle, high reproduction rate along with a tolerance to shifting temperature and moisture conditions as well, a global distribution. They have good tolerance to changing environmental conditions due to their natural occurrence in the organic soil horizon where temperature and humidity can alter more than in the deeper horizons. There we instead find the anecic and endogeic species of earthworms that resides deeper in the soil profile, who makes burrows vertically and horizontally, respectively (Wong et.al 2020).

3.4.2. Wastes for Vermicomposting

The organic wastes suitable for vermicomposting are many. To name a few; manure from animals, human food waste and food industry waste, paper industry waste, residues from agricultural or horticultural crops and even sewage sludge and solids from wastewater, will work to sustain an earthworm population (Dominguez & Edwards 2010). Another benefit from vermicomposting wastes is a significant reduction of volume. A vermicomposted pig slurry was reduced from 1 m^3 volume of 80% moisture to half, 0.5 m^3 with 30% moisture (Sinha et.al 2010).

3.4.3. Treatment and Application

Vermicomposting differs much from other controlled composting methods. The latter normally reaches *thermophilic* temperatures, 55˚C while at vermicomposting, temperatures are kept at *psychro-* or *mesophilic* range, approximately not more than 45˚C. The higher temperatures results in heat-tolerant groups of microbes where other soil organisms are being ruled out. Because of the aerobic condition and a steady controlled temperature, vermicomposting displays both a higher diversity and number of microorganisms (Sherman 2018, Dominguez 2010). A food web is exhibited in the vermicompost, consisting of microorganism and soil invertebrates whom interacts to perform a collaborative cycling of organic material similar to natural decay (Dominguez & Edwards 2010). Millions of decomposer microbes reside in earthworms gut only and, these are excreted from the gut together with organic material that have been grinded by the earthworm's gizzard. The biowastes used in vermicomposting can be referred to as feedstocks however, worms are really not feeding on these, but rather on the microorganisms involved in the decomposition of the organic material (Sherman 2018, Dominguez 2010). The microorganisms host the enzymes to perform the actual biochemical decomposition and the earthworms are indirectly driving this process by breaking up and ingesting the organic material, resulting in smaller pieces which increase the surface area of organic material (Dominguez & Edwards 2010). This increased surface area supports microbial colonization and thus increase the decomposition rate. Moreover, microbial species in the mentioned gut flora are dispersed through worm castings (vermicast) and the tunneling of earthworms in the organic material aerates the environment, enhancing activity of microorganisms (Wong et.al 2020).

Parameters Influencing the Vermicomposting

Like with any decomposing process the carbon to nitrogen ratio (C:N ratio) of the organic material is important, because being the feed for decomposing microorganisms this ratio affects the rate of degradability. The C:N ratio is preferably 25-30:1, similar to composting (Arancon et.al 2010, Sherman 2018). There are other physical and environmental factors that affect this decomposition process. Particle size, structure/rigidity, bulk density and porosity are physical factors of the feedstock affecting degradability and environmental conditions such as temperature, oxygen, pH, moisture also influences the process.

Content in Vermicompost

In a vermicomposting process nutrients are mineralized and become plant-available while any contaminants, such as heavy metals and pathogenic bacterial strains are significantly reduced (Lotzof 1999 see Sinha et.al 2010). Total coliforms can be reduced with as much as 98% compared to a fresh pig slurry when passaging through the guts of earthworms (Monroy et.al 2008, 2009 see Dominguez 2010). Vermicompost is rich in bioavailable inorganic nutrients N, K and P, where N particularly is in nitrate and ammonium-form (Dominguez 2010). Other reported components are micronutrients, beneficial soil microorganisms, humic substances (Arancon et.al 2010) and plant hormones (Wong et.al 2020). Herein the humic substances and plant hormones will be discussed under section Bioactive Ingredients and Plant Response.

Application

There are a few products from a vermicompost and as well a few different ways of application. A common way is to substitute the vermicompost with another compost, soil medium or field soil (Atiyeh et.al 2001, Arancon et.al 2008). Another product from vermicomposting is the collected leachate, a liquid that can accumulate and leak during the decomposition process (Quaik & Ibrahim 2013). Singh et.al (2010) performed foliar spraying of leachates from different vermicomposts, every 30 days for five months on field grown strawberries. The leachates were collected from three vermicomposts; one from cow dung, second from vegetable waste and thirdly, a mixture of 1:2 cow dung to vegetable waste. The results showed increased leaf area, dry matter of plants resulting in better quality of the berries and improved marketable fruit yield. The mixture derived-leachate, of cow dung and vegetable waste, had highest increased marketable fruit yield with 26.5%, due to a reduction of albinism, malformation of fruit and grey mold. Showing that quality of produce may be enhanced by biostimulant application.

Another liquid product is making vermicompost tea, an aqueous solution. This is produced by a brewing process, where solid vermicompost is mixed with water and then aerated for some time with the aim to multiply beneficial microorganisms from the vermicompost. This results in water extracts containing microorganisms, soluble nutrients, and plant-beneficial substances from the solid vermicompost (Salter & Edwards 2010) such as plant hormones (Wong et.al 2015, 2020).

3.4.4. Bioactive Ingredients and Plant Responses

Already in 1963 researchers on earthworms hypothesized that effects from worm castings on soil fertility was due to bacterial polysaccharides increasing soil aggregate stability and an enhanced rate of breakdown since the organic matter is processed through the earthworms gut and associated microbes (Parle 1963 see Tomati et.al 1988). Other researchers, with beginning in 1959, concluded that the N solemnly released from the earthworms wasn't enough for plant nutritional needs (Barley & Jennings 1959; Aldag & Graft 1975; Dash & Patra 1979 See Tomati et.al 1988). This made them imagine that mechanisms for the plant growth effects from earthworms are explained by something else than nutrient availability and physical characteristics in the soil.

Arancon et.al (2008) tested three different vermicomposts for growing petunias in greenhouse. These three were made from cattle manure, paper waste and food waste and substituted at different proportions with a commercial growing medium. Results from bioassay showed that all of them proved significant increases on both growth rate and amount of root and shoot dry weight as well as germination rate. The increase of root and shoot growth rate was considerably higher at lower substitution proportions than at higher ones, where slower growth rate started to show at 70% substitution in all three different vermicomposts. Whereas a 40% substitution rate proved successful for all three. This decrease was thought to occur by the plant hormone auxin and humic acids' restrictive effects at high concentrations, and referred to previous research (Hopkins and Huner 2004, Arancon et.al 2006b see Arancon et.al 2008). In this experiment the petunias nutritional needs were accommodated equally for all treatments rejecting nutrient availability as a factor responsible for the increases in growth. Therefore the beneficial effects on germination, growth and flowering of petunias, were proposed to relate with plant growth influencing substances namely, humic acids and plant hormones and the increase of beneficial microbes; their population, diversity and in turn their own production of enzymes and hormones.

Plant hormones and hormone producing microorganisms

Today there are proof that vermicompost contains plant hormones and/or plant-growth promoting substances. The hormones detected are cytokinin, auxin, gibberellin and brassinosteroids (Wong et.al 2020). They together make up a hormonal network ruling the faith of many physiological processes in plants. Cytokinin and brassinosteroids have been described in the seaweed section but gibberellins and auxin will be touched upon here.

The gibberellins have a part in cell elongation and cell division together with e.g. cytokinin. Moreover, stem elongation, formation of flowers, leaf expansion, germination and development of seeds are also traits belonging to gibberellin (Wong et.al 2020, Evert & Eichhorn 2013).

Auxin regulates various processes related to plant growth and development. At the cellular level, like cytokinin, auxin has a role in the cell cycle checkpoints. Additionally these two, auxin and cytokinin, and the ratio between them will determine promotion of either root or shoot growth (Wong et.al 2015). When a balance occur between them, cells are kept undifferentiated but at a higher cytokinin : auxin ratio shoot development is promoted and the opposite promotes root development.

Vermicompost has, as mentioned, a high number and diversity of microorganisms. This diversity can improve the suppressive-ness of a soil, meaning better resistance towards invasion from pathogenic microorganisms. This can be explained by a decrease of resources due to a diversity that occupies majority of the niches as well as the resources leaving less habitat for invasive species (Turner et.al 2013). Moreover RGPR (root growth promoting rhizobacteria), can change the chemical composition of root exudates, transforming them into plant hormones that the plant can take up and utilize (Marschner 2012).

Humic substances

Humic substances can be called by-products of microbial decomposition, made up of many different-sized molecules from microbial residues and transformed organic matter. This group of compounds are naturally created in soil when organic material degrade, resulting in a reservoir of organic C and N (Madende & Hayes 2020), accounting a big part of the soil organic matter (SOM) (Bot & Betaine 2005). Humic substances found in soils have been reported to contain proteins, carbohydrates, open-chained biopolymers, and lignin making them supramolecular (Calvo et.al 2014). Meaning humic substances are large, carbon dense molecules comprised of many smaller molecules. Biostimulant effects from humic substances are commonly related to architecture of plant roots where increased root size, root branching and higher density of root hairs been documented (Zandonadi et.al 2007, Canellas et.al 2015,). Other seen effects have been increase water stress tolerance, decrease plant disease rates, reduced rates of fertilizer applications and enhanced earlier growth and flowering (Halpern et.al 2015).

Humic substances that are added to crop production systems today are mainly sourced from coal and peat, being non-renewable resources (Canellas et.al 2015). A sustainable alternative for humic substances are instead derived from the degradation of organic wastes. Degradation of organic material in vermicomposting produces humic substances which in turn influence the bioavailability and mobility of metals. Humic substances have this ability to form stable complexes, with metal micronutrients due to their O, N and S functional groups, thereby making them organo-bound metals (Wong et.al 2020, Madende & Hayes 2020). These humic substances are furthermore hypothesized to create aggregate-stability by bounding to eg. clay minerals and absorb plant growth hormones which are released gradually at a rate well attuned to plant growth (Arancon et.al 2010). There are three categories of humic substances, divided into *humic acid*, *fulvic acid* and *humin*, based on their solubility at certain pH (Cannelas et.al 2015). The latter is insoluble in any pH, fulvic acids soluble at all levels and humic acids only soluble at higher pH levels.

Extracted humic acids from a pig manure vermicompost, mixed with a peat based medium at several different application rates were tested on tomato and cucumber (Atiyeh et.al 2002). Rate of growth including plant height, leaf area, and shoot and root dry weight increased when the concentration of humic acids were between 50 to 500 mg humic acids / kg −1. However, at concentrations above 500 mg humic acids / kg −1 there was significant decrease of the same parameters. This pattern of decrease of plant growth due to high concentrations are, as mentioned throughout this thesis, also found for vermicompost and several other biostimulants. It is reported that vermicomposts made with animal manure, sewage sludges or paper-mill sludges contains humic and fulvic substances in large amounts (Albanell et.al 1988, Petrussi et.al 1988, Senesi et.al 1992, Garcia et.al 1995, Masciandaro et.al 1997, Elvira et.al 1998 See Arancon et.al 2010). This may be appraised if one seeks to extract humic substances from a vermicompost for separate application. Atiyeh et.al (2001) extracted 4 g of humic acids per kg of vermicompost using an acid/alkali fractionation technique.

The ability of humic substances to lower pH of root surfaces and nearby soil is affecting plants' nutrient uptake and tolerance to stress. When humic substances are applied, the activity of an enzyme in the cell membrane of the plant have been seen to increase. This enzyme is called H+-ATPase (Madende & Hayes 2020, Calvo et.al 2014), and is also referred to as proton pump, because it is creating a proton gradient where an excess of protons on one side of the cell membrane allows substances to be "pumped" through to the inside. In other words this electrochemical gradient is creating energy that in turn drives processes of secondary metabolites and nutrients to move through the cell membrane (Canellas et.al 2015). With other words; the permeability of membranes of root cells increases (Atiyeh et.al 2002). This furthermore creates a more effective absorption of nitrate, since nitrate can move together with protons through the cell membrane (Halpern et.al 2015).

A property of humic substances that seem most associated with root growth promotion was its degree of hydrophobicity (Canellas et.al 2015). Since humic compounds are supramolecular, they have varying charges connecting these smaller molecules together. These charges alters the hydrophobicity of the molecule. This means, that humic supramolecular structures may not be disrupted by water, however, the organic acids that microorganisms create and plants exude by their roots might have the power to do so (Calvo et.al 2014, Canellas et. al 2015). This would indicate that the effectiveness of humic substances in crop production stand in relation to plant root exudation, and microorganisms creating organic acids, acting as the chemical force which breaks apart the bioactive molecules in humic substances. The bioactive molecules that can be released from humic superstructures can perform hormone-like activities on plants (Wong et.al 2020), and there may even be several bioactive molecules that's not yet identified, inducing hormone-like activities (Zandonadi et.al 2007). Jindo et.al (2012) noted, from application of vermicompost-derived humic acids on maize roots, enhanced root growth and a proliferation of lateral root emergence along with an enhanced proton pump activity (H+-ATPase). They could also declare that exchangeable auxin was in the structures of the humic acids, perhaps hosting the beneficial root growth. Another conclusion they drew, similar to Canellas et.al (2015), was that higher hydrophobicity was linked to more bioactivity.

The mechanisms of humic substances causing increased nutrient uptake are thus related to processes in soil; from improving nutrient solubility in soil, increasing aggregate stability and regulating pH in the rhizosphere. And there are mechanisms directly effecting plant physiology aiding the increased nutrient uptake, such as the expression of roots and the activity of the proton pumps which increases nitrate absorption.

3.4.5. Mineral Nutrient Content in Vermicompost

The mineral nutrient content in vermicompost depends on the used feedstock. Table 6 demonstrates content of some primary mineral nutrients in finished vermicomposts, where different biological wastes have been decomposed.

Table 5. Mineral nutrient content in vermicompost made from different biowastes, where Nitrogen (N), Phosphorus (P) and Potassium (K) are shown in % of dry matter, Calcium (Ca) and Magnesium (Mg) shown in microgram per gram -1. (Adapted from Wong et.al 2020)

Macronutrient	**N**	**P**	**K**	**Ca**	**Mg**
Vermicompost and Type of waste	**% DM**	**% DM**	**% DM**	**Microgram g -^{1}**	**Microgram g -^{1}**
Food waste	1,30%	2,70%	9,20%	18614	4364
Paper waste	16	1,40%	6,20%	9214	7661
Yard waste	1,56%	0,40%	2,65%	-	-
Sheep manure	1,18%	0,00%	-	-	-
Cow manure	1,35%	0,70%	1,28%	3600	-

That concludes the section on Vermicomposting, where the process and its resulting products have been described along with two of the bioactive ingredients (plant hormones and humic substances) and some plant responses.

4. DISCUSSION

4.1. The Challenging Situation

Food production systems need to increase its efficiency to feed humanity's increasing population. Tremendous yield-increases were achieved after the 2nd world war, by means of mechanical development and large inputs of agrochemicals – two operations that relies on non-renewable fossil fuels (Waterlow et.al. 2000). The effects on the environment from some agri- and horticultural practices have not been as bright as the increased yields. Excess input of mineral fertilizers, increased input of pesticides and soil-disturbance have had detrimental effects on terrestrial and marine ecosystems (Tilman et.al 2001, Tedengren 2021). The use of fossil fuels has increased greenhouse gases in the earths' atmosphere, which have great influence on climatic patterns. Climate change has begun to, and is estimated to keep increasing abiotic and biotic stresses on plants (Tuteja 2012, Dutta et.al. 2020, Vaughan et.al. 2018).

4.2. Inputs for Plant Production

Successful yields are not only determined by size of the harvest, but stands in relation to what it took you to create that yield. High-input systems are estimated to achieve better yield than low-input systems, but what resources did it take to produce that yield and what consequences did they generate? Excessively used mineral fertilizers, has had detrimental effects to environment (Tilman et.al 2001, Tedengren 2021), and accumulation of agrochemicals are becoming a problem in ecosystems (Naccarato et.al 2020). In addition, some of the main limiting nutrients to plant growth such as phosphorus are becoming increasingly expensive to mine thus, pushing up prices which leads to global socioeconomic inequality (George et.al 2016). This scenario of a need for improved yields, accumulated agrochemicals, with climate change posing great challenges to plant growth is pressing agricultural and horticultural practices to (*i*) become non-polluting, (*ii*) use renewable material and, (*iii*) in general become more efficient with its inputs of water, fertilizers and pesticides. Plant biostimulants seems to be one toolbox, containing a multitude of different organic compounds and microorganisms, able to impact plants in such a way that can improve plant growth, increase plants tolerance to stress and nutrient efficiency while being produced from renewable by-products.

Thus, the goal for plant production isn't just about yield-increases but also to reach good quality and nutritional value of food, and moreover, to achieve this in terms of economic, social and environmental sustainability. According to me, sustainability is to make better use of resources. EU suggests that a circular bio-economy is a sustainable concept. The core process that will determine a successful bio-economy is photosynthesis. This process is mainly performed by plants and is driving the production of feed, food and fibre which are the material for bio-based products and biofuel. Circularity is to make the best use of resources for as long as possible. Within this concept there would ideally not exist any wastes, but only by-products.

4.3. The Biowastes as Potential Biostimulants

Examples of by-products suitable as biostimulants have been mentioned throughout this thesis with the main focus held on seaweeds, fish waste and the processing of various biowastes through vermicomposting. It turns out that seaweeds have long been acknowledge for its beneficial effects as a soil amendment and now, in the shape of seaweed extracts also as a biostimulant.

With fish waste, the by-products from industry is expected to increase (FORMAS 2021). Sweden has initiatives such as; "Blue Food - a centre for the seafood of the future" which are in pursuit of becoming a leading producer of seafood, connecting science, government and industry (Aquavitae 2021).

As for vermicomposting, the benefits of dealing with biowastes in such a process are numerous; biosafety of the wastes are improved without requiring chemicals to kill off pathogens; bulk density of the biowastes decrease; the process creates humified organic matter with high content of beneficial microorganisms, bioactive compounds and nutrients with high bioavailability. And moreover, a variety of different biowastes can be processed in a vermicompost both on small and large scale, with high or low technology set ups (Dominguez & Edwards 2010). Making vermicomposting a process suitable for agricultural by-products, industrial by-products from timber industry or food processing facilities and, as well urban wastes such as sewage sludge. Moreover, it requires low technology and low energy to produce vermicompost because biology is doing the work. Therefore I believe monitoring of the decomposition process and analytical work is what will be costly.

The biostimulants from these mentioned by-products can improve many aspects of plant productivity. For example increased nutrient uptake due to increased mobility or solubility of minerals; amino acids from FPH may chelate mineral nutrients (Madende & Hayes 2020), humic substances from vermicompost increases nutrient bioavailability (Wong et.al 2020) and seaweeds are recorded to stimulate mineral nutrient uptake (Crouch et al. 1990). Another psychological trait shown from biostimulant-applications is increased length and density of roots, which can lead to improved nutrient uptake. Plant growth can generally be enhanced by some biostimulant's content of plant hormones or precursors, and organic inputs in general may support microbes who can produce plant hormones and enzymes benefitting the plant, which is the case of vermicompost (Arancon et.al 2008, Wong et.al 2020).

Moreover, the impact biostimulants can have on plants' stress responses are sought after, especially if extreme weather events are becoming more common due to climate change. The results presented in this thesis bring about a few of the aspects in which biostimulants promote plant tolerance to abiotic stresses. For example by adjusting osmotic pressure in cells to avoid denaturing (osmoprotectants), inducing antioxidant systems to protect from oxidative damage and influencing the hormonal crosstalk network resulting in better responses to environmental stress response. Biostimulants mode of action are still hard to elucidate, and will most likely be an ongoing subject for many years to come. Where knowledge between fields of science need to be shared for a faster development of effective biostimulants. Table 6 summarizes some of the traits of each biostimulant sourced from biowastes that has been mentioned herein. Note that the mineral nutrient content is sourced from the already mentioned tables (table 2, 4 and 5) which are examples found in the literature I've collected, these are most likely not representative but used a examples.

Table 6. Summary of traits of each biostimulant from the biowastes mentioned in this thesis.

Biostimulant	**SWE**	**FPH**	**Vermicompost**
Primary mineral nutrient composition	Per litre (N) - (P) 43 mg (K) 52mg (Ca) 1060 mg (Mg) 406 mg	Per kilogram (N) 55 g (P) 100 g (K) 0,006 g (Ca) 182 g (Mg) 3 g	Per kilogram (N) 13 g (P) 27 g (K) 92 g (Ca) 18,6 g (Mg) 4,4 g
Main group of bioactive compound	Carbohydrates + plant hormones	Proteins	Humic substances + plant hormones + beneficial microorganisms
Plant hormones	Auxin Cytokinin, Gibberellin Abscisic acid Ethylene Brassinosteroids Jasmonates	-	Auxin Cytokinin Gibberellin Brassinosteroids
Treatment	- Enzymatic hydrolysis - Extraction (acid, alkali or water) - Ultrasound-assisted extraction - Enzyme-assisted extraction - Supercritical fluid extraction - Microwave-assisted extraction - Pressurized liquid extraction	Hydrolysis of fish parts - Chemically - Enzymatically	Decomposition process of biological wastes

4.4. The Complexity of Biostimulant's Biological Nature

It seems rather clear that it is the organic fraction, and the composition of many different compounds and/or microorganisms, that creates the desired plant responses from biostimulants most likely in synergy (Yakhin et.al 2017, Ali et.al 2021). Neither ashing or separate fractions of SWE showed the same effects as a complete SWE, and the general mineral nutrient content in biostimulants is not considered enough to elicit the benefits recorded. Frankenberger & Arshads' (1995) conclusions, that organic material are more effective than a sterilized medium and that organic material displays psychological effects which cannot be replaced by an equal amount of nutrients, supports the idea that the organic fraction in biostimulants performs synergistic effects in plants and their rhizosphere, not seldom mediated by microbes. Thus, biostimulants can act directly on plants or indirectly through the microorganism community.

The ability of organic compounds to transform depending on environmental conditions such as pH, temperature, UV-radiation, makes the manufacturing of biostimulants complex and the shelf-life uncertain. This might be one of the greater challenges for the biostimulant market; to be able to guarantee the content of a biostimulant product and even more, the exact mechanisms of the plant responses. Because most biostimulant products are heterogenous, meaning that the content differs. In Europe, biostimulants were first registered in 2019 through the Fertilizer Product Registration (FPR), and the European Biostimulant Industry Council announced that the first CE-marked biostimulant can be placed on the market in July 2022 (EBIC 2021). This will according to EBIC make it possible to at least, ensure that it is a safe product for humans, animals and environment. Working out the mechanisms of biostimulants are an important, challenging and continuous task in the hands of research. But until then, biostimulants can be considered a safe product.

I believe that farmers who implement biostimulants in their crop management by treating their on-farm biological wastes into biostimulants may be rewarded. If not by enhanced quality of plants, increased stress tolerance or better nutrient efficiency then there are likely indirect effects on plants performed by the soil microorganisms who can feast on organic compounds such as proteins, carbohydrates and humic substances.

4.5. Biostimulants and Mineral Nutrients

Often the beneficial responses from biostimulants don't reach the same levels when sufficient mineral nutrients are lacking (Crouch et.al 1990). Which leads me onto a study reviewing 14 meta-analyses concerning organic inputs role on soil organic matter (SOM) content and its relation to yield-increases (Hijbeek et.al 2018). Within this work they took into account the methods used in each meta-analysis and the most interesting, (to make my point) was that the effect of macronutrients, N, P and K, was included in half of them and excluded in the other half of the studies. One conclusion they drew which caught my attention was that in the cases where macronutrients were accounted for, meaning the treatments received the required levels of macronutrients, the results from organic inputs were all positive. But when the macro nutrients weren't supplied equally there was much fewer positive correlations between SOM levels and yield. What I see, which corresponds to the lack of response in plant growth from biostimulants applied without the nutrient requirements, is that organic inputs in general may not be sufficient without covering the plants' need for mineral nutrients. SOM includes an active organic fraction still going through decomposition and includes living microorganisms, making up about 10-40%, and the other part is 40-60% of more stable organic matter (Bot & Betaine 2005). Microorganisms need mineral nutrients in their metabolism too, leading to immobilization of nutrients which generally results in a delay of mineralization of nutrients (Wong et.al 2015). Being a reason of organic fertilizers unpredictability of delivering plants the required nutrients at the required time.

This leads me to the answer on my final research question on what gives most successful yields; the usage of one or several biostimulants. The biological material utilized as biostimulants have different origin with different characteristics (e.g. mineral nutrient content, pH, bioactive substances) giving each biostimulant an area of capability. Where SWE mineral nutrient profile looks different to FPH and vermicompost. They can differ in amount and type of plant hormone content, where SWE and vermicompost has them and FPH lacks them. Suggesting that a mix of FPH (rich in amino acids=high N and P) may be suitable to combine with vermicompost (rich in humic substances+hormones) and/or SWE (rich in K, micronutrients, carbohydrates, hormones). Using a combination of biostimulants might therefore fulfill the holistic requirements of plants and associated beneficial microorganisms, both because of their content but also because of the effects this content has on nutrient uptake. Such as, chelating effect of amino acids and SWE, humic substances ability to bind minerals and, microbes in vermicompost mineralizing nutrients in the soil. This is a suggestion which might lead us closer to a circular bioeconomy, where recycling of renewable resources can limit the dependency on mineral (chemical) fertilizer inputs seen today.

Moreover, the treatment of biological wastes do not require expensive measures but can simply be performed by the microorganisms decomposing the organic material. The process needs to be monitored if one wishes to control e.g. moisture, pH, temperature to shorten the time of decomposition. But if facilities are designed accurately, in terms of keeping a certain climate suited for decomposition, all it takes is some time for biology to do its thing. Lastly, I want to press that this knowledge, of adding organic material to crop-cultivation, isn't something new in human history. But what is new are the "omics" technologies (genomics, transcriptomics, proteomics, or metabolomics) helping us understand HOW the organic compounds affects soil microorganisms and plants. These analytical tools are on the other hand expensive but required if biostimulants are to fit a regulatory framework.

4.6. The Increased Need for Biofuel and Biobased Products

With a growing realization of fossil fuels detrimental influence on climate change, many governments are shifting to renewable alternatives. This may have an impact on the whole food production system and thus, determine the economic possibility to reclaim nutrients and foremost, biostimulants from biowastes, back to plant production. Azar (2005) explained one economic challenge; *"The more costly carbon-free energy could raise energy prices to a level that would mean higher profits for the bioenergy sector. With these higher profits, farmers would have greater economic incentives to turn to bioenergy, unless food prices rose to the point where profits matched the energy sector. Thus, land and food prices are likely to be pushed upwards."* (Azar 2005:99). This possible scenario makes me wonder if the desired energy residing in biowastes, might be more profitable than the potential biostimulants therein.

Fish waste and seaweeds are suggested as material for biobased products, as food additives, and as active ingredients in pharmaceuticals (Madende & Hayes 2020, Wu et.al. 2019). This interest for biobased products might create a demand which will push prices up. This is all speculation. But I do believe, biobased and circular economies will develop the necessary measures for transforming wastes into valuable resources and that the demand for these resources is going to lead the development forward. Perhaps, within a functioning circular bioeconomy these biological resources can be fully utilized by all interests.

5. CONCLUSIONS

- Plant biostimulants seems to be one toolbox that suits both organic and mineral fertilizer systems, that contains a multitude of different organic compounds and microorganisms, able to achieve better plant growth, increase plants tolerance to stress and nutrient efficiency while being produced from renewable by-products.

- Plant biostimulants sourced from biological wastes are heterogenous. Because of the varied organic content in the wastes and the chosen treatment there are variations in the nutrient composition and the amount and type of bioactive substances.

- Plant biostimulant products are applied at low concentrations and benefit plants regardless of nutrient content. Suggesting that it is the organic fraction primarily responsible for the effects on plants. Although they contain mineral nutrients, biostimulants are by definition not considered enough to fulfill the plants nutrient requirements.

- In the future, mixtures of biostimulants from biowastes may aid the holistic nutrient requirement of plants, if we can analyze the content and match the application to the plant’s requirements. Indicating that analytical costs might be what determines the wider use of biostimulants in plant production.

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Appendix 1

Table 7. Listing of examples of bioactive compounds in SWE within different categories and group of macroalgaes. (Adapted from Ali et.al 2021)

	Mega class of Seaweed		
Category	**Brown**	**Green**	**Red**
Carbohydrates	Alginates Cellulose Heteroglucans Fucose Fucoidans Glucuronoxylofucans Laminarans Lichenan-like glucan	Amylose, amylopectin Cellulose Glucomannans Inulin Laminaran Ulvans Sulfated mucilages Xylans Pectin Mannans	Agars, agaroids Cellulose Mannans Carrageenans Complex mucilages Furcellaran Glycogen (floridean starch) Xylans Rhodymanan
Protein, amino acids, peptides	Histidine, Isoleucine, Leucine, Lysine, methionine, Phenylalanine, Threonine, Tryptophan, Valine, Cysteine, Arginine, Aspartic acid, Glutamic acid, Alanine, Glycine, Proline, Serine, Tyrosine and Alanine -Taurine -α-Kainic acid	Histidine, Isoleucine, Leucine, Lysine, methionine, Phenylalanine, Threonine, Tryptophan, Valine, Cysteine, Arginine, Aspartic acid, Glutamic acid, Alanine, Glycine, Proline, Serine, Tyrosine and Alanine -Taurine -Domoic acid -α-Kainic acid	Histidine, Isoleucine, Leucine, Lysine, methionine, Phenylalanine, Threonine, Tryptophan, Valine, Cysteine, Arginine, Aspartic acid, Glutamic acid, Alanine, Glycine, Proline, Serine, Tyrosine and Alanine -Taurine -Domoic acid -α-Kainic acid
Lipids	Glycolipids, Betaine lipids, Non-polar glycerolipid, (neutral lipids), Unusual lipid class	Glycolipids, Betaine lipids, Non-polar glycerolipids (neutral lipids), Mannose and rhamnose	Sulfur-containing phospholipids, Phosphatidyl sulfocholine, Glycolipids, Betaine lipids,

		containing glycolipids	Non-polar glycerolipids (neutral lipids), Sulfonoglycolipid crassiculisine
Minerals	Macro (C, Cl, Fe, Mg, P, K, Na and S) -Micro (B, Cr, Co, Cu, F, Gr, I, Mn, Mo, Ni, Se, Si, S, Tn, W, V, Zn)	Macro (C, Cl, Fe, Mg, P, K, Na and S) -Micro (B, Cr, Co, Cu, F, Gr, I, Mn, Mo, Ni, Se, Si, S, Tn, W, V, Zn)	Macro (C, Cl, Fe, Mg, P, K, Na and S) -Micro (B, Cr, Co, Cu, F, Gr, I, Mn, Mo, Ni, Se, Si, S, Tn, W, V, Zn)
Plant growth regulators	Cytokinins Auxins Gibberellins Abscisic acid (ABA) Indole-3-acetic acid (IAA) Ethylene Brassinosteroids Jasmonates Salicylic Acid Strigolactones Zeatin Kinetin 6-benzyl amino purine (BAP)	Cytokinins Auxins Gibberellins Abscisic acid (ABA) Indole-3-acetic acid (IAA) Ethylene Brassinosteroids Jasmonates Salicylic Acid Strigolactones Zeatin Kinetin 6-benzyl amino purine (BAP)	Cytokinins Auxins Gibberellins Abscisic acid (ABA) Indole-3-acetic acid (IAA) Ethylene Brassinosteroids Jasmonates Salicylic Acid Strigolactones Zeatin Kinetin 6-benzyl amino purine (BAP)