MICROALGAE AS A BENEFICIAL SOIL AMENDMENT

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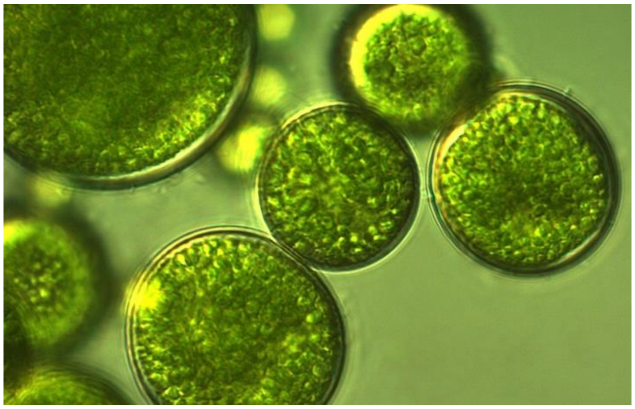

Summary:

This white paper provides technical in-depth perspective of the agricultural uses of microalgae and their benefits to both soil health and a variety of crops. Before jumping into the content, our author gives a brief overview of microalgae including their diversity, various commercial applications in different markets, and how they are cultivated.

About the Author:

Dr. Kris Nichols is a leader in the movement to regenerate soils for healthy soil, crops, food, people, and a planet. She is a Soil Microbiologist with over 25 years of research experience studying arbuscular mycorrhizal (AM) fungi. Kris builds upon a soil health foundation to identify biological methods for agricultural production and tools and practices to reduce pest issues, soil erosion, fossil fuel use, and greenhouse gas emissions. Throughout her career, she has given over 250 invited presentations to a wide variety of audiences, authored or co-authored more than 25 peer-reviewed publications, been cited or interviewed for more than 50 magazine or newspaper articles, highlighted in five books, and has numerous videos on-line. Dr. Nichols was the Chief Scientist at Rodale Institute for over three years. Prior to joining Rodale Institute, Dr. Nichols was a Research (Soil) Microbiologist with the USDA, Agricultural Research Service (ARS) in North Dakota for 11 years and a Biological Laboratory Technician with ARS in Beltsville, MD for 3 years. Kris received Bachelor of Science degrees in Plant Biology and in Genetics and Cell Biology from the University of Minnesota in 1995, a Master's degree in Environmental Microbiology from West Virginia University in 1999, and a Ph.D. in Soil Science from the University of Maryland in 2003. Kris grew up on a 640-acre grain farm in SW Minnesota which is still managed by her father, Jim Nichols who was a former State Senator and Commissioner of Agriculture.

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TABLE OF CONTENTS

EXECUTIVE SUMMARY

Algae are ubiquitous on Earth in both aquatic and terrestrial environments from the tropics to the arctic. These organisms are 2.4—3.6 billion years old and can tolerate a variety of conditions including a wide range of pH levels, temperatures, salinity, and light intensities. Microalgae are a diverse group of algae ranging in size from a few to a few hundred microns and being both autotrophic and heterotrophic. In agriculture, these organisms are used as biofertilizers, biopesticides, and plant growth promoters (PGPs). Specifically, microalgae enhance soil health; improve water management; increase resilience against stressors such as pests, drought, or salts; and add fertility by making macro- or micro-nutrients in soil available or acting as a direct source of fertility.

Soil structure improves with microalgae because the mucilaginous sheaths, exopolysaccharides, hydrophobic biomolecules, and other microbial substances create soil aggregates. Increased aggregation boosts porosity for enhanced aeration, water infiltration, and water holding capacity. Soil aggregates, microalgal cells, and microalgal exudates and other biomolecules are partially comprised of carbon and build soil organic matter. Since microalgae are highly efficient at photosynthesis and replicate rapidly to large numbers, soil organic matter and soil fertility rise. Microalgal cells and biomolecules contain large amounts of plant nutrients. In addition, nitrogen fixation and phosphorus solubilization by some microalgae provide inorganic nitrogen and phosphorus to plants.

Microalgae enhance plant resilience against soil salinity or sodicity, pests, stressors, or climate by producing various carbohydrates, proteins, enzymes, fatty acids, organic acids, vitamins, hormones, and other biomolecules. These biomolecules immobilize salts, alter pH levels, or reduce salt absorption by plants. Bacterial, fungal, viral, protozoal, and nematode pests are controlled these biomolecules as well as by physical protection of foliar or root tissue when mucilaginous sheaths prevent pest's access to plant cells. This physical protection as well as improvements in soil aggregation and biomolecules that absorb water provide resilience against drought, UV radiation or temperature extremes.

By working alone or in conjunction with other microorganisms such as nitrogen-fixing bacteria, mycorrhizal fungi, or phosphate-solubilizing bacteria, microalgae boost foliar and root growth as well as crop productivity. This plant growth promotion results from the biochemistries of one or more microalgal biomolecules as well as the benefits conveyed from better soil health, fertility, and resilience.

Ongoing public and private research on utilizing microalgae in agriculture will continue to optimize the design of this system to fully actualize the benefits described for microalgae for centuries. To increase the density of microalgal cells and/or biomolecules, microalgae are cultured in open or closed systems and applied to cropland. A system, such as the one designed by MyLand Company LLC, produces robust microalgae in high concentrations and applies live algal cells via streamlined injection into irrigation systems.

WHAT, WHY AND HOW

What are Microalgae?

Microalgae are a group of unicellular auto- and heterotrophic microorganisms ranging in size from a few to hundreds of microns and found in a variety of agro-ecosystems across the globe. Chlorophyta (green algae), Rhodophyta (red algae), Phaeophyta (brown algae), Euglenophyta, Pyrrophyta, Chrysophyta, and Cyanobacteria are microalgae phyla classified by pigmentation, life cycle and structure (Win et al., 2018). The number of microalgal species are estimated to be in the hundreds of thousands with population densities in topsoil ranging from 3 and 100 million cells per gram (Chiaiese et al., 2018).

Due to their versatile nature – simple unicellular structure, high photosynthetic efficiency, ability for heterotrophic growth, adaptability to domestic and industrial wastewater, and production of biomolecules – microalgal use is being implemented in agriculture (Chiaiese et al., 2018). Chlorella spp., Dunaliella spp., Haematoccocus spp., Isochrysis spp., Nannochloropsis spp., Porphyridium spp., Spirulina spp. and most cyanobacteria are the microalgae used most in industry (Chiaiese et al., 2018). In agro-ecosystems, microalgae have been primarily used in rice, but also in grains such as wheat, corn, sunflowers, peas, and chickpeas; cotton; sugar beets; sugarcane; produce crops such as lettuces, cabbage, peppers, tomatoes, and radish; ornamental flowers and trees; and other crops. Studies have shown increases in plant root/shoot length, dry weight, yield, germination rate, cellular respiration, floral production, photosynthesis, chlorophyll production, and/or resilience to pests, diseases, and climate stressors (Mahapatra et al., 2018; Ronga et al., 2019). Overall, rice yields have increased by up to 30% with microalgal addition (Mahapatra et al., 2018; Uysal et al., 2015). This yield increase has occurred primarily due to cyanobacteria performing free-living and symbiotic atmospheric nitrogen gas fixation. The fixed nitrogen, ammonia, may be converted into polypeptides, free amino acids, vitamins, and auxin-like substances which add fertility either by secretion or through microbial degradation after cell death. Studies have shown that this nitrogen fixation may account for up to 100 kg N ha^{-1} yr^{-1} (Warren, 2014).

Although microalgae possess chlorophyll-a and have carbohydrate, protein and products comparable to those of higher plants, they also are able to grow heterotrophically on a variety of carbon sources such as glucose, glycerol, ethanol, and volatile organic acids (Chalima et al., 2017). These microorganisms have a wide range of physiological and biochemical characteristics and contain up to 50-70% protein, 30% lipids, over 40% glycerol, and 8-14% carotene, vitamins, and minerals (Priyadarshani and Rath, 2012). These biomolecules act as food sources for other microbes and become macro- and micronutrients or function to promote plant growth. Exopolysaccharides (EPSs) produced by microalgae are integral to soil health by binding small soil particles together into soil aggregates. Increasing soil aggregation is elemental to expanding porosity which impacts water use efficiency – water permeability or infiltration and water retention. Mucilaginous sheaths and EPSs also allow microalgae to survive desiccation even up to 70 years in storage (Trainor, 1970). The intercellular and extracellular biomolecules produced by microalgae act as antibacterials, antifungals, antiprotozoals, antivirals, (Kumar et al., 2019) and nematicides (Singh et al., 2016).

Typically, solar radiation, water, and temperature are the most important abiotic factors governing microalgal distribution, metabolism, and life strategies. Wastewaters are frequently used for culturing microalgae because they are an inexpensive source of necessary nutrients needed for microalgal algal growth (Mahapatra et al., 2018). In these systems, toxic and xenobiotic compounds are broken down and nutrients are mineralized. Both the living and dead algal cells from these systems are applied to crops, seeds, foliar tissues or soil with or without other products. Following application, microalgal cells will rapidly colonize the rhizosphere to enhance plant growth and development. From a fertility perspective, microalgae primarily have been exploited for the nitrogen fixing capabilities which has focused most of the research on cyanobacteria. However, contributions to phosphorus solubilization and chelation of micronutrients such as iron and calcium have gained prominence (Meeting, 1981). Some microalgal species are capable of tolerating pesticides, particularly insecticides (Win et al., 2018) while others reduce heavy metal toxicity (Hussain and Hasnain, 2011).

In addition to uses in agriculture, microalgae have been used in industry as bioreactors or living-cell factories to produce bio-fuels and numerous fats, sugars, and bioactive compounds used in food, aquaculture, poultry, pharmaceutical, and cosmetic industries (Kumar et al., 2019; Priyadarshani and Rath, 2012). Research studies in these areas have expanded scientific understanding of the beneficial roles of biomolecules produced by microalgae in soil health, pest management and reducing the impacts of climatic stressors such as UV radiation, desiccation, and temperature extremes. These factors may also be linked to the activities of microalgae in biocrusts which initiated much of the research exploring microalgae in soils.

Microalgae in Biocrusts – Microalgae along with mosses, lichens, and fungi are components of 2mm to 2 cm biological crusts found in mesic and arid ecosystems (Ferrenberg et al., 2015; Isichei, 1990; Liu et al., 2013; Warren, 2014)). These crusts can constitute up to 70% of dryland ground cover and are a substantial portion of primary production (i.e. 6.4 to 370 kg C ha^{-1} yr^{-1}) (Warren, 2014). Biocrust organisms are adapted to limited moisture and low nutrient conditions and even tolerate high salt conditions. Early successional biocrusts are dominated by cyanobacteria and are more prone to erosion, dust production, and reduced water infiltration, which can have complex, long-lasting effects on local ecosystem processes. As successional processes continue filamentous cyanobacteria, algae, and fungi, as well as rooting structures of lichens and bryophytes increase soil structure (Abdel-Raouf, 2012). Overall, soils under biocrusts had 11.3-fold higher organic matter, 10.4-fold higher total N, 3.25-fold higher C:N ratio, 1.46-fold higher total P, 1.57-fold higher soil moisture, and 1.13-fold lower daytime temperatures than uncrusted soil (Liu et al., 2013).

Why Apply Microalgae?

Despite these microorganisms being used as biofertilizers for centuries, research only has been conducted on algae's impacts on productivity and yield since the early 1950s following the development of mass culturing techniques (Win et al., 2018). However, over a 10-year period, research studies on algae as biofertilizers are only about 0.04% of the studies published on agriculture or 0.09% of studies published on algae (Mahapatra et al., 2018). The majority of these studies explored the activities of prokaryotic cyanobacteria rather than eukaryotic algae primarily due to the nitrogen fixation abilities of these microbes. Microalgae can be applied to the soil, foliar tissue, or seed. Isochrysis spp., Chaetoceros spp., Chlorella spp., Arthrospira spp. and Dunaliella spp. are the algal species most used in industry with Arthrospira spp. and Chlorella spp. being the main cultivated microalgae species.

Microalgae are of interest to agribusinesses and farmers due to their biofertilization, plant growth promotion (PGP) and/or biopesticide properties which increase agricultural sustainability and enhance resilience (Uysal et al., 2015). The following roles have been described for microalgal even though the specific mechanisms have not been fully identified:

- Solubilizing and/or mobilizing macro- and micronutrients
- Acting as a source of organic matter and nutrients
- Complexing heavy metals and xenobiotics to limit their mobility and transport into plants
- Mineralizing simpler organic molecules such as amino acids for direct uptake by plants
- Protecting plants from pathogens and diseases
- Buffering pH
- Enhancing resilience against stressors such as drought or salts
- Stimulating plant growth
- Improving the physico-chemical conditions of soils, i.e. forming soil aggregates
- Provide oxygen to subsurface areas

Soil Health — Soils, particularly those in deserts and semi-arid regions, frequently are highly compacted, low in fertility, saline or sodic, poorly aerated, retain less water, and easily eroded (Uysal et al., 2015). In these soils, microalgae make physio-chemical contributions to soil health by assisting in the formation and stabilization of soil aggregates which increase pore space and continuity. This in turn improves water infiltration and water holding capacity, aeration, nutrient cycling and seed germination (Isichei, 1990). Microalgae physically entwine soil particles with multicellular filaments or by biochemically attaching organic matter, sand, silt or clay to their mucilaginous cellular sheaths or exopolysaccharides (Harper and Belnap, 2001). Also, chelators form organo-mineral complexes to enhance aggregation by binding metals such as calcium, iron and zinc as well as aluminosilicate clay minerals. The accumulation of soil particles in aggregates create nutrient-rich microsites where the sheaths are coated with negatively-charged clay particles which then bind positively-charged nutrients (e.g., iron, copper, molybdenum, zinc, cobalt, and manganese) reducing leaching of these nutrients from the soil profile (Harper and Belnap, 2001). In addition to sheaths and biomolecules, *Chlamydomonas* sp. can effectively colonize abiotic surfaces such as fine sand particles via flagella-mediated adhesion and electrostatic interactions to further soil aggregation (Kreis et al., 2019). Soil aggregation following microalgal application to a sandy loam, loam, and a silty clay loam increased by 85%, 130% and 160%, respectively (Kaushik, 2014). Organic compounds exuded by microalgae or microalgal cells themselves are constituents of soil organic matter which is essential for soil health (i.e. more sustainable and/or higher crop production) (Abdel-Raouf, 2012).

Organic Matter — Microalgae are important sources of organic matter since their photosynthetic rate is about 10-50 times faster than in terrestrial plants (Uysal et al., 2015). Some of these biomolecules as well as microalgal cells contribute significantly to sequestered soil organic matter because their cell walls contain recalcitrant algaenans (Derenne and Largeau, 2001). These biomolecules protect algal cell walls, particularly at the soil surface, and are highly aliphatic and not easily decomposable. Microalgal PGPs also are organic matter constituents, help to improve biochemical reactions and act as a carbon source (Ronga et al., 2019). For example, *Spirulina* spp. cells are about 86% organic compounds – proteins (54%); carbohydrates (23%); and lipids, vitamins, enzymes, etc. (8.6%) (Kumar et al., 2019). Nutrient status of soil specifically nitrogen and phosphorous determines the mineralization of available carbon and thus affects the microbial community (Chatterjee et al 2017).

Climate Resilience and Mitigation — Increases in organic matter by the application of microalgae plays an important role in climate resilience and mitigation. Although microalgae frequently are classified as indicators of climate issues rather than potential mitigators, particularly in response to nonpoint source water pollution and water temperatures increases (e.g. algal blooms). Commercial and industrial production of microalgae mitigate climate change by:

1. Absorbing carbon dioxide as part of the photosynthetic reaction.
2. Replacing the high carbon footprint of chemicals used in the pharmaceutical and cosmetic industries by using microalgae as bioreactors to produce these chemicals (Kreis et al., 2019; Ronga et al., 2019).
3. Storing carbon in the soil by increasing organic matter (Derenne and Largeau, 2001; JaiPaul et al., 2014; Kumar et al., 2019; Tripathy and Singh, 2004).
4. Reducing nitrous oxide emissions in agriculture, particularly in rice production, by replacing nitrogen fertilizer with biological nitrogen fixation (Tripathi et al., 2008).
5. Increasing water infiltration rates and water-holding capacity improves water use efficiencies to reduce the carbon footprint of irrigation systems (Lewis et al., 2019; Priyadarshani and Rath, 2012).
6. Replacing fossil fuels with highly efficient algal-based biofuels.
7. Enhancing fertility and pest management with organic biomolecules rather than synthetic, fossil fuel-based chemicals (Uysal et al., 2015).

Soil and Plant Fertility — Since microalgae contain the same twenty macro- and micronutrients needed by plants for growth and development, the bodies of these organisms themselves are a source of fertility. Therefore, macronutrients – carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) – and the micronutrients – iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), copper (Cu), zinc (Zn), nickel (Ni), chlorine (Cl), sodium (Na), cobalt (Co) and selenium (Se) concentration increase with microalgae growth and production. In addition, microalgal biomolecules contain many of these nutrients as well. Table 1 below illustrates that a particular microalga (*Spirulina* spp.) contains nutrient-rich biomolecules as well as higher levels of N, P, K and Ca than organic fertilizer but lower levels of NPK than chemical fertilizer (Mahapatra et al., 2018). Similarly, *Arthospira* spp. contains 6.70, 2.47, and 1.14% N, P and K, respectively (Ronga et al., 2019). The slow-release form of organic fertility found in microalgal biomass (i.e. lipids, carbohydrates and proteins) stimulates primary and secondary metabolites and intracellular transporters in plants that result in greater root development (i.e. higher values for total and fine root length and greater numbers of root tips) which enhances nutrient uptake from the soil (Uysal et al., 2015).

Table 1. Comparison of the macromolecular and chemical composition of the microalga *Spirulina* sp. cultured in media-based bioreactor or from wastewater-fed lakes to organic and chemical fertilizer.

Macromolecular composition	*Spirulina* sp. (cultivated)	*Spirulina* sp. (wastewater -fed lakes)	Organic fertilizer	Chemical fertilizer
Lipids (%)	11	9.8	-	-
Carbohydrates (%)	22	18.2	-	-
Proteins (%)	48.13	45	-	-
Nitrogen (%)	7.7	7.2	1.09	12.4
Phosphorus (%)	0.88	0.71	0.7	6.6
Potassium (%)	1.76	2.44	1.27	12.5
Calcium (%)	0 .67	0.52	0.48	0.1

Microalgae directly enhance soil and plant fertility by providing or enhancing the availability of nutrients in the inorganic forms. Because cyanobacteria are capable of nitrogen fixation, they are a ready source of N (Kumar et al., 2019; Tripathi et al., 2008). Nitrogen fixation is highly dependent on water and light, as well as species composition, with maximum fixation at approximately 26°C and above 20% moisture. Five to 88% of N fixed by *Nostoc* has been shown to leak into the surrounding substrate with 11-16% of the total nitrogen being mineralized (Harper and Belnap, 2001). Other microalgal activities have been found to stimulate the availability of other inorganic nutrients such as P, Fe, Cu, Mo, Zn, Co, and Mn. Microalgae have been found to increase the bioavailability of phosphorus to the plants at levels higher than from synthetic fertilizers (Uysal et al., 2015). Phosphorus solubility is increased by microalgae through the formation of biomolecules which chelate calcium and release phosphate from calcium phosphates, the exudation organic acids to reduce soil pH and solubilize phosphate, and/or synthesis of phosphatase enzymes (Uysal et al., 2015). Chelation also enhances the availability of micronutrients such as Fe, Cu, Mo, Zn, Co, and Mn along with binding negatively-charged nutrients such as phosphates, nitrates, and sulfates in an exchangeable form to the chelated cations on microalgal sheaths (Chatterjee et al., 2017).

In unfavorable soil conditions, such as high or low pH, high salinity or high calcium carbonate ($CaCO_3$) levels, both macro- and micronutrients are less likely to be available due to competitive binding and immobilization. Microalgal biomolecules can ameliorate these unfavorable conditions through the production of organic acids, enzymes, and/or competitive chelators. Additional fertility loss may occur with erosive forces and climatic issues (i.e. drought and high temperatures). For example, in desert ecosystems, nitrogen concentrations are known to be low because up to 77% of the nitrogen may be lost through wind erosion, ammonia volatilization, nitrification, and denitrification (Harper and Belnap, 2001). Improvements in soil aggregation with microalgae as described above reduces erosion and nutrient loss while better water management, as outlined below, may lower volatilization and immobilization.

Salinity and Sodicity — Globally, nearly one billion hectares of soil are affected by salinization (Chatterjee et al., 2017). Salinity can negatively impact soil physical and chemical properties resulting in increased soil compaction and erosion. Excess salinity affects approximately 20% of irrigated arable land and is responsible for damage to plant development, especially at the seedling stage. The deleterious effects of salinity on plant growth are associated with plant metabolism, nutrient deficiencies, osmotic stress, specific ion toxicities, or a combination of these factors (Kumar et al., 2019). Once inside plant cell, salts can cause ionic stresses, largely by Na (and Cl) inhibiting major processes such as photosynthesis, protein synthesis, and energy and lipid metabolism. The following mechanisms are used by microalgae to tolerate salinity and sodicity (Chatterjee et al., 2017; Kumar et al., 2019; Uysal et al., 2015):

(1) producing of extracellular polysaccharides to buffer salts

(2) releasing organic acids through microbial decomposition of organic matter to react with calcium carbonate, release calcium and form carbonic acid

(3) utilizing chelators to bind and immobilize calcium and sodium

(4) synthesizing and accumulating osmoregulatory compounds such as sugars and quaternary amines to impart high osmotic tension to plant roots for absorption of water and nutrients

(5) maintaining low internal sodium by either restricting uptake or efflux through algal biomolecules or in consortia activities of microalgae with other rhizosphere microorganisms

(6) substituting calcium or potassium ions for sodium ions in clay complexes

(7) improving permeability, aeration and water movement through soil aggregation

(8) expressing of a set of salt-stress responsive proteins

Water Management — After cell division, microalgae leave behind sticky, mucilaginous sheaths which connect soil particles to form soil aggregates. Soil aggregates are less susceptible to wind and water erosion than their constituent parts and increase porosity. Greater levels of porosity increase water infiltration rates and water-holding capacity as well as better soil aeration. Studies of biocrusts have found that moistened sheaths absorb ten times their volume in water (Colica et al., 2014; Fan et al., 2008; Johansen et al., 1984; Warren, 2014). Although in most cases the sheaths and other biomolecules including exopolysaccharide increase water holding capacity due to their hygroscopic (i.e. ability to attract water molecules) nature, hydrophobicity (i.e. water-hating) in some of these molecules or high clay content can contribute to surface sealing (Singh et al., 2016). Too much or too little soil disturbance of biocrusts via grazing, traffic or cultivation may restrict pathways for water flow and reduce water availability by altering the concentrations of cyanobacteria, other microalgae, lichens, and mosses (Colica et al., 2014).

Humidity and water levels impact microalgal biological processes. For example, photosynthetic activity can be higher at low water levels while nitrogen fixation may be reduced (Aranibir et al., 2003). These differences make the impacts of microalgae on plants very complex. For example, in non-water-stressed tomato plants, microalgae stimulated better root length and an increased leaf number and leaf area while in water-stressed tomato plants, alleviation of water stress increased plant height (Ronga et al., 2019).

Pest Management — Microalgae form antibacterial, antifungal, antiprotozoal, nematicides, and antiviral compounds such as exopolysaccharides, hydrophobic biomolecules, and hormones

(Kumar et al., 2019). The types of antimicrobial compounds vary greatly, and their production depends on the composition of culture medium, incubation period, pH, temperature, and light intensity during culturing. of these antimicrobial compounds. The antibacterial activity varies depending on the microalgal species with more hydrophobic biomolecules such as polyketides, amides, alkaloids, fatty acids, indoles, and lipopeptides being most effective (Ronga et al., 2019). Similar compounds also have antifungal activities particularly against soil-borne or foliar fungal pathogens (Singh et al., 2016). Saprophytes—*Chaetomium globosum, Cunninghamella blakesleeana*, and *Aspergillus oryzae*, and plant pathogens such as *Rhizoctonia solani* and *Sclerotinia sclerotiorum* decrease with microalgal application because of some specific antifungal compounds having been isolated (Kumar et al., 2019). Additional antimicrobial activities come of biophysical protection of plant foliar tissue and roots by mucilaginous sheaths and biofilms produced by microalgae (Kumar et al., 2019). Specifically, studies have found cell constituents of cyanobacteria, likely through biophysical protection, reduced the incidence of *Botrytis cinerea* on strawberries, *Erysiphe polygoni* powdery mildew on turnips and damping off disease in tomato seedlings (Singh et al., 2016). In grains, *Fischerella muscicola* showed antifungal activity against *Uromyces appendiculatus* (brown rust) and *Pyricularia oryzae* (rice blast). *Nostoc muscorum* has displayed antifungal against soil fungi and especially those producing “damping off” and *Sclerotinia sclerotiorum*, or “white mold,” in lettuce, vegetables and other species of rosette plants (Singh et al., 2016). Following microalgal application nematode egg hatching decreases and immobility and mortality of juvenile plant parasitic nematodes increases (Win et al., 2018). Also, in desert ecosystems, biocrusts containing microalgae reduce exotic annual grass germination to reduce invasive species (Liu et al., 2013; Warren, 2014).

Plant Growth Promotion –– Microalgae produce a variety of biomolecules which act as plant growth promoters (PGPs) and impact cellular respiration, photosynthesis including leaf chlorophyll, nucleic acid and antioxidant synthesis, and ion uptake in plants (Ronga et al., 2019). These PGPs include auxins, cytokinins, betaines, amino acids, vitamins, polysaccharides and polyamines (Ronga et al., 2019). In some cases, gibberellins, brassinosteroids, protein hydrolysates, and amino acids can be extracted from microalgal cultures and applied via sprays (Win et al., 2018). These PGPs enhance plant growth and development, mitigate injuries caused by abiotic stresses, and influence metabolic activities such as root and shoot development, leaf senescence, breaking bud dormancy, seed gemination, photosynthesis, respiration, nucleic acid synthesis, and nutrient uptake. In addition, amino acids such as tryptophan and arginine impact the growth and yield of cultivated crops because these two amino acids are the metabolic precursors of aromatic secondary compounds, polyamines, and plant hormones such as auxin and salicylic acid (Chiaiese et al., 2018). These compounds impact embryogenesis, flower initiation and development, fruit setting, ripening and leaf senescence. Other plant growth promoters include cytokinins and indole-3-acetic acid (IAA) enhance growth, yield, and nitrogen concentrations (Hussain and Hasnain, 2011) while photoprotective compounds/pigments are including mycosporine-like amino acids (MAAs), scytonemin and carotenoids protect against UV radiation, oxidative stress, osmotic pressure, thermal stress and are typically used in cosmetics (Kumar et al., 2019). Significant increase in soil polysaccharides, dehydrogenase, urease, and phosphatase activities also have been measured with microalgal application.

Interactions with Other Organisms –– When microalgae are combined with nitrogen-fixing, phosphate-solubilizing and/or other plant growth promoting microorganisms their benefits to plants are enhanced (Uysal et al., 2015). Mycorrhizal fungi and *Rhizobium* bacteria coupled with microalgae stimulate greater levels of rhizosphere activity, nutrient cycling and soil aggregation (Harper and Belnap, 2001). Phosphate-solubilizing bacteria – *Bacillus megaterium, Bacillus circulans, Bacillus subtilis, Bacillus mucilaginous* and *Pseudomonas striata* – act in consortia with microalgae to mobilize phosphate (Mahapatra et al., 2018).

How are Microalgae Produced and Applied?

Microalgae are cultured in bioreactors such as open ponds, pits, or tanks, or closed photobioreactors for application to cropland or extraction of biomolecules (Lewis et al., 2019; Priyadarshani and Rath, 2012; Ronga et al., 2019). In open systems, the growing medium is directly exposed to the air where evaporation helps to regulate temperature but light limitations, aeration, water to replace evaporative loss and contamination algal grazers are issues. One way in which open systems reduce costs is using nutrient-rich wastewater as the culturing medium which also provides a mechanism to reduce heavy metal (i.e. lead, nickel, and cadmium) toxicity in the wastewater (Ronga et al., 2019). The most popular type of open system are raceway ponds with a depth between 10 and 50 cm, to allow for appropriate illumination, and having a paddle wheel for gas/medium mixing and circulation. A closed system or photobioreactor focuses on optimizing light capture and use a tubular or flat-plate design while controlling evaporation, culturing media, contamination, and temperature. Typically, photobioreactors have higher volumetric productivity and can better capture light than open ponds which rely on natural light. Table 2 below describes differences between a photobioreactor or closed system and a raceway pond or open system (Narala et al., 2016). *Arthrospira* spp., *Dunaliella* spp., *Anabaena* spp., *Phaeodactylum* spp., *Pleurochrysis* spp., *Chlorella* spp. and *Nannochloropsis* spp. are typically cultured in open systems while closed systems culture *Porphyridium* spp., *Phaeodactylum* spp., *Arthrospira* spp., *Nannochloropsis* spp., *Chlorella* spp., *Haematococcus* spp. and *Tetraselmis* spp. (Narala et al., 2016; Ronga et al., 2019).

Table 2. The factors involved in utilizing a system for culturing microalgae are compared between a photobioreactor or closed system and a raceway pond or open system.

Factor	Photobioreactor	Raceway Pond
Space required	Moderate	High
Evaporation loss	Low	High
CO2 Sparging efficiency	High	Low
Maintenance	Difficult	Easy
Contamination risk	Low	High
Biomass quality	Reproducible	Variable
Energy input for mixing	High	Low
Operation type	Batch	Batch
Setup cost	High	Low
Maintaining continuous	Difficult	Difficult

exponential phase

Table modified from Narala et al. (2016).

In agriculture, microalgae convey numerous benefits as described in the above sections and are applied as living or dead cells using sprayers, liquid injectors, or irrigation system to the soil, foliar tissue, or roots. If foliar-applied, microalgal extracts are readily absorbed by the leaf through its stomata and cuticle pores, showing the best effectiveness if applied in the morning, when stomata pores are completely open (Chiaiese et al., 2018; Mahapatra et al., 2018; Ronga et al., 2019). Therefore, plant responses to nutrients supplied via foliar sprays are normally more rapid than when applied as a soil amendment.

Downstream processing is utilized to either convert the liquid cultures into a solid or to make a microalgal extract which contains the beneficial biomolecules. Although the density of beneficial solids, extracts, or compounds may be higher, the costs associated with downstream processing are at least 20-30% of the total production cost (Silva and Silva, 2013). Techniques for creating these products include centrifugation, drum drying, and lyophilization or freeze-drying to create a solid algal product (Ronga et al., 2019). For efficacy of microalgal extracts to optimized the cells need to be lysed or cell walls ruptured, this is done by concentrating the microalgal cells with centrifugation or flocculation or clumping cells together with clays, crop residue, or fly-ash and gravity sedimentation followed by cryo-freezing with liquid nitrogen and grinding by a mill, treating with high pressure followed by a pressure reduction or using enzymes such as protease, cellulase, β-glucosidase, xylanase, β-glucanase, and pectinase (Ronga et al., 2019; Win et al., 2018). If flocculating agents are added to cultures to concentrate microalgal cells, these carriers are capable of increasing the shelf-life of the microalgal biofertilizer (Win et al., 2018). Although these solid products may have longer shelf lives than liquid products, both the solid microalgal cells or the solidified extracts need to be suspended in water for application.

SUMMARY AND CONCLUSIONS

Benefits of Microalgae as a Biological Amendment

Naturally, microalgae are found in a variety of systems from aquatic to arid environments. The biofertilizer, PGP and biopesticide properties of microalgae have been found to enhance soil organic matter accrual, water use efficiencies, rooting, higher crop yields and quality, and tolerance to pests, drought and salts (Chatterjee et al., 2017; Derenne and Largeau, 2001; Mahapatra et al., 2018; Priyadarshani and Rath, 2012; Ronga et al., 2019).

These activities are enhanced when using microalgae in agriculture as a biological amendment. Although research has been limited to date, microalgae have been applied as amendments in cropping systems, particularly in rice production, for over 2000 years. The economic benefits of microalgal application may not always come from increases in production but from savings in fertility expenses and benefits to ecosystem services. Studies have found boosts in plant shoot and root growth, grain or fruit production, and in fruit or grain quality but results have not always been consistent and additional study is needed. Also, microalgae can induce tolerance to biotic and abiotic stressors, including pests and diseases, drought, or salts. Summaries of the current status for marketing microalgae as a biofertilizer and/or PGP, research studies, and the development of an on-farm bioreactor system designed by MyLand Company LLC.

Marketing Microalgae

Marketing microalgae has focused primarily on use in pharmaceuticals and cosmetics, biofuels, and feed for animals including humans and is gaining traction in agriculture. As a rich source of macro- and micro-nutrients and carbon-based biomolecules such as polysaccharides, proteins, enzymes, vitamins, etc., microalgae are becoming increasingly more marketable in agriculture.

There are currently a few companies – Heliae®, AlgEternal®, Ferticell®, TrueAlgae®, and AgriAlgae® – marketing microalgae or microalgal based products, primarily *Chlorella vulgaris,* in agriculture. All of these companies are marketing products which contain either living - AlgEternal® - or pasteurized - Heliae® - organisms or extracts of microalgal biomolecules - Ferticell®, TrueAlgae® and AgriAlgae®. In some cases, the products are amended with bacteria, enzymes and minerals and be sold as either liquid or dry products. If the products do not contain living microalgae, they will have a longer shelf-life, but they do not provide the benefits gained from living organisms. The pasteurized or microalgal extracts both have additional downstream processing costs which limit their large-scale marketability in agriculture. Both the dry and liquid products are diluted in water and are applied through irrigation, sprayed into the seed bed or on foliar tissue, or as a bare root or transplant drench. See the bulleted lists below for more information about these companies:

- **Heliae**®
    - Products – **PhycoTerra**® or **PhycoTerra**® **Organic**
    - Composition – liquid suspended 10% solids product of pasteurized *Chlorella vulgaris*
    - Activity – Food for other rhizosphere microorganisms to release beneficial biomolecules
    - Application - Mixes in water and applied planting in seed furrow, onto seedbed, sidedress, or as a bare root or transplant drench; through irrigation; or sprayed directly
    - Storage – at room temperature for a week or refrigerated with vented cap for up to 12 months
- **AlgEternal®**
    - Product – **ElixEarth**® **Soil Amendment Concentrate (Previously Agtivate®)**
    - Composition – Liquid concentrate of living *Chlorella vulgaris* and *Scenedesmus acuminatus* - 10-15 million cells per milliliter (mL) and ~60% water
    - Application - 1 gallon diluted for application on 10 acres with sprayer, irrigation system, or watering can and apply to soil when damp or before rains in furrow, on foliar tissue or by soaking seeds one day before planting
    - Storage – at room temperature for a week or refrigerated with vented cap for up to 12 months
- **Ferticell**® (Previously Agroplasma®)

    - Products – **Universal 0-0-1, Calcium 880, Microelements 1-0-0** and **Nutri-Plus 2.5-0-0,**
    - Composition – All products contain microalgal extracts mixed with other elements such as potassium sulfate, calcium carbonate, amino acids, with each specifically derived to a particular function.
    - Activity – **Universal 0-0-1** – increased root development and better flowering and fruit growth
        - **Calcium 880** – stimulates cell elongation to improve nutrient, particularly nitrogen uptake, and help alleviate sodium issues in the soil to enhance porosity
        - **Microelements 1-0-0** – increased chlorophyll content, enzyme function, and water absorption for drought tolerance
        - **Nutri-Plus 2.5-0-0** – induces protein synthesis for recovery from stress or pollen formation and fertility
    - Application – Apply via irrigation with **Nutri-Plus 2.5-0-0** recommended before, during or after stress event
    - Storage – Products are stable for up to 2 years
- **TrueAlgae®**
    - Product – **Chlopia-P Liquid Fertilizer**
    - Composition – liquid product derived from Chlorella Vulgaris
    - Activity – **Chlopia-P Liquid Fertilizer** – stronger root development, enhanced seed germination rates, improved water absorption, yields and plant vigor
    - Application – apply through irrigation water or spray on leaves after diluting at 1:200-250
- **AgriAlgae®**
    - Product – multiple **AgriAlgae Original, AgriAlgae Enhanced, AgriAlgae Premium,** and **AgriAlgae Organic** products supplement with nutrients such as copper, iron, or potassium
    - Composition- Pasteurized Chlorella
    - Activity – increased yields and fruit development and quality
    - Application – Applied to soil or leaves

The MyLand Difference

Unlike other companies marketing microalgae, MyLand Company LLC (MyLand) in Phoenix, AZ is not marketing microalgae or a microalgal product but rather is marketing a unique photobioreactor system (Myland System) to produce indigenous microalgae and service for this system. The MyLand System is designed to produce high-density cultures of microalgae which are then applied to crop fields at the grower's desired rate and frequency, for example, via irrigation system – flood, drip, or sprinklers. In addition to not selling a product, the MyLand difference utilizes indigenous microalgae cultured from soil and/or water samples collected from individual sites and applying live cells. Win et al. (2018) found that indigenous species of *Anabaena* spp. were successful in promoting soil fertility while reducing compaction and were more resilient to herbicides and drought. By utilizing indigenous algae, the organisms will be adapted to the soil textural and climatic conditions that impact microalgal growth, activities and interactions with other soil organisms.

The MyLand System is designed to apply live algae in a high density to enhance soil and plant health and address issues limiting crop production. The system uses specialized photobioreactors called Algae Production Vessels (APVs). MyLand is currently on the 3.0 version of the APVs and has progressed from around 10 trillion cells per day to about 50 trillion cells per day by optimizing lighting, aeration, and culturing media. The impacts of microalgae cultivated with the MyLand System on crop production and soil health are currently being examined through on-farm research at a farm near Buckeye, AZ – River Ranch – and through controlled pot culture and research farm studies established in 2019. Preliminary data from these studies is summarized below.

Preliminary MyLand Data

River Ranch

In 2015, an on-farm study was initiated for an alfalfa crop at the River Ranch farm. Soil, water and alfalfa plant samples were collected in the spring and the following issues were identified:

- Low soil organic matter levels (2.43%) even after multiple applications of human sludge,
- Low Relative Feed Value for alfalfa particularly in the hot summer months,
- High levels of sodium in the soil and high pH, and
- Low levels of beneficial microbiological density and diversity.

By August 2016 the MyLand 2.0 System was fully operational with a portion of field 17N (the "**Control Field**") designated to act as a control site and the field identified as 16N being treated with algae (the "**Treated Field**"). This system produced algae at a rate of 9.3 trillion cells per day (3 million cells per mL of output, at 75% uptime). Over the next few years of operation, hardware and software enhancements were implemented resulting in an increase in output to 11.55 trillion cells per day (5 million cells per mL of output, at 75% uptime). However, by early 2019, the System 2.0 was replaced with MyLand System 3.0 which can produce 68.82 trillion cells per day (25 million cells per mL of output, at 92% uptime).

Over time, soil pH decreased from 8.03 to 7.8 along with a 16.9% decrease in calcium levels from 6500 to 5400 ppm while magnesium and potassium increased slightly from 340 to 350 ppm and from 180 to 240 ppm, respectively, in the Treated Field. In the Control Field, pH increased slightly from 8.14 to 8.3 while calcium, magnesium, and potassium levels decreased from 7400 to 5900 ppm, 520 to 439 ppm, and 210 to 190 ppm respectively. In the Treated Field, nitrates, phosphates, sulfur, and potassium increased from 10 to 66 ppm, 45 to 54 ppm, 110 to 190 ppm and 180 to 240 ppm, respectively. Observations by the farmer found larger, moister, and more lush alfalfa leaf tissue; faster alfalfa regrowth after haying; lower additional fertilizer needs, and less crusting requiring less tillage with microalgal application. In a response to the positive preliminary results and observations indicated with the MyLand System, controlled research studies were established in 2019.

MAC Study

MyLand initiated a controlled research study in fall 2019 at the University of Arizona- Maricopa Agricultural Center (the MAC study). The study hypothesis is that microalgae will improve soil health and crop productivity and quality. A subterranean drip irrigation system was established in an ~4-acre field with capability to test up to five algal treatments and four crops. In the drip system, most of the microalgae added will be growing heterotrophically or will be food for other microbes and receive multiple treatments.

This study is in its nascent stages and as with many field studies, there were issues with irrigation system and natural pests such as rabbits and birds. However, preliminary data collected does indicate that algal treatment improve lettuce crop production, particularly for romaine. Data is still be collected and analyzed on two other crops harvested this winter, broccoli, and celery, as well as soil data. Ongoing work is being conducted to repair and optimize the irrigation system for better water and algal distribution. Finally, spring crops – cantaloupe, watermelon, tomato, and pepper – were planted on March 24, 2020 representing our first spring season crop trial.

Conclusions

Microalgae have been a component of agricultural systems for centuries and since the 1950's these organisms have been specifically applied to cropping systems. Research studies on microalgae as a biological amendment in agriculture are continuing to increase year after year and the data already confirms these microorganisms enhance soil health and crop production and quality. Higher yields have been recorded with microalgal application as well as increases in protein, vitamin, and antioxidant concentrations in fruits and grains. Soil health parameters such as organic matter levels, macro- and micronutrient content, and soil aggregation have increased while heavy metal toxicity and salinity or sodicity issues have been ameliorated.

Utilizing microalgae as a biological amendment has a lot of potential not only from the benefits described above but also because they have the capability to grow both photoautotrophically and heterotrophically and a wide diversity of species. Cyanobacteria have the capacity to fix atmospheric nitrogen which provides additional fertility particularly in desert ecosystems where available nitrogen is limited. Finally, microalgae have high concentrations of proteins, carbohydrates, lipids, vitamins, minerals, and other beneficial biomolecules. Some of these biomolecules may be exuded during algal growth and act as hormones or other plant PGPs or as aggregators or glues (i.e. exopolysaccharides) in aggregation. These characteristics make microalgae a strong candidate to be marketed to agribusinesses. As the research increases from both academia and the private sector, there is no question that microalgae are not a gimmick but play a key role in soil health with regenerative properties for depleted soils.

APPENDIX A.

Glossary

Algaenans are resistant biopolymers in the cell walls of green algae.

Aliphatic compounds are comprised of hydrocarbon chains and are not easily dissolved in water.

Autotrophic organisms produce their own food using light, water, carbon, and/or other chemicals. **Photoautotrophs** use light as the energy source for producing food while **chemoautotrophs** use chemical such as methane as their energy source.

Biofertilizers are products containing living microorganisms or natural substances able to improve chemical and biological soil properties, stimulate plant growth, and restore soil fertility.

Biostimulants are organic products such as hormones that stimulate the growth and development of crops under both optimal and stressful conditions.

Chelator is a molecule that binds metal ions. The bonds created by chelators vary in strength and weak chelation may make the metal ions exchangeable or available for sharing bonds to bind anions weakly to the metal cations.

Exopolysaccharides are high-molecular-weight biopolymers produced during the growth or propagation of microorganisms including microalgae. These compounds are excreted from the cells into the surrounding environment or be loosely attached to the cell wall.

Exudates are molecules released by organisms outside of the cell wall or cell membrane into the surrounding environment.

Flocculants are substances that clump molecules together so they settle out of solution by gravity more easily.

Heterotrophic organisms eat other organisms either living or dead organisms as their energy and nutrient source.

Hydrophobic molecules repel water and are fats or oils typically found in membranes and on surfaces.

Hygroscopic molecules attract and hold water molecules from the surrounding environment.

Mucilage is a thick, gluey coating made from carbohydrates, proteins and/or lipids on the surface of cells that may be hydrophobic.

Photobioreactor is a manufactured device or system that uses light to cultivate photoautotrophic organisms.

Plant-Growth Promoter (PGP) is a natural biomolecule, typically an organic acid, hormone, or vitamins that stimulate plant growth.

Plant Growth Regulator (PGR) is a natural or synthetic plant hormone that alter the growth or physiological processes of the plant.

Recalcitrant compounds resist decomposition or degradation.

Salinity containing high concentrations (i.e. the electrical conductivity or salt content is 4 mmho cm^{-1} or more) of salt ions such as sodium, calcium, potassium, magnesium or chloride. In saline soils, clay particles are dispersed and clog pores which reduces hydraulic conductivity and aeration.

Sodicity occurs when sodium ions dominate over other salts in saline soil which can raise soil pH to 10 and impact soil structure even further.

Soil Aggregate is clod or conglomeration where sand, silt and clay particles as well as organic matter adhere to each other more strongly than the surrounding environment. The adhesion comes from ionic binding mostly between cations and organic matter, microbial including microalgal polysaccharides, and/or hydrophobic molecules.

Soil Health is the continued capacity of soil to function as a vital living ecosystem that sustains plants, animals and humans.

Xenobiotics are foreign or unexpected and in some cases unnatural compounds.

APPENDIX B.

Research Study Results

Below is a summary from some relevant research results:

1. *Dunaliella* spp. and *Phaeodactylum* spp. extracts mitigated salt stress during the seed germination process of bell pepper by the formation of organic biomolecules which reduced superoxide radical production and peroxidation (Uysal et al., 2015).
2. *Spirulina* spp. and *Chlorella* spp. were applied to wheat to improve tolerance to salinity through the stimulation of higher antioxidant and protein content (Uysal et al., 2015).
3. *Chlorella vulgaris* increased germination height and rate in wheat and corn plants (Uysal et al., 2015).
4. Chlorella pyrenoidosa cultured in dairy waste-water effluent increased root and shoot length in rice by 30% (Win et al., 2018).
5. *Anabaena laxa* increased chickpea yield by 50% (Win et al., 2018).
6. Sugar and carotenoid concentrations increased in tomato fruits treated with dry biomass of *Nannochloropsis* spp., *Ulothrix* spp. and *Klebsormidium* spp. (Uysal et al., 2015).
7. Seedling growth was improved with a significant enhancement of soluble carbohydrate, soluble protein and total free amino acids from *Chlorella vulgaris* applied to lettuce seeds. When application was made to soil, there was an increase in fresh and dry weight as well as pigment content (Uysal et al., 2015).
8. When *Acutodesmus dimorphus* was applied to pots 22 days prior to transplanting tomato seedlings, there were more branches and flowers than with a no algae control. Similar studies showed faster germination rates when used as a seed primer and increases in plant height and a greater number of flowers and branches after foliar application (Uysal et al., 2015).
9. In rice, *Nostoc* spp., *Hapalosiphon* spp., and *Aulosira* spp. application improved seed germination, root and shoot growth, weight of rice grains and protein content. Research showed that these benefits came from the presence of root-promoting hormones such as auxins, cytokinins and gibberellic acid (Uysal et al., 2015).
10. *Arthrospira* spp. was foliarly applied every two weeks to red beet (*Beta vulgaris* L.) in an organic cropping system and resulted in higher dry and fresh weights compared to the control (Uysal et al., 2015).
11. Following foliar application of *Arthrospira* spp. and *Scenedesmus almeriensis* extracts at 10 g L^{-1} at 0, 14, 28, 35, and 42 days after transplanting on ornamentals, there was higher rates of root, leaf and shoot growth, earlier flowering, more flowers per plant, and higher plant water content (Uysal et al., 2015).
12. *Arthrospira* spp. applied as a foliar spray on tomato and pepper plants at different growth stages improved plant size by 20 and 30%, respectively, while the effects on root weight were more marked in tomato (+230%) than pepper plants (+67%). Also, the size and number of nodes per plant improved after the treatment by 57% and 100% and 33% and 50% in tomato and pepper, respectively. No microalgal extracts displayed phytotoxicity problems (values greater than 50%) on germination.
13. *Calothrix elenkenii* controls damping off by the soil-borne plant pathogen *Rhizoctonia solani* (Singh et al., 2016).

14. *Fischerella muscicola* reduces brown rust, powdery mildew and rice blast (Singh et al., 2016).
15. *Nostoc muscorum* negatively impacts cottony rot and damping off by the soil-borne plant pathogen *Rhizoctonia solani* in vegetables and flowers (Singh et al., 2016).
16. Under optimal conditions, *Nostoc* could produce 8.66 lg/ml IAA, and sprouting in a taro field was effectively promoted (Win et al., 2018).
17. A gibberellin-like plant growth hormone was produced by *Scytonema hofmanni* have produced gibberellin-like plant growth regulators enabled the hormone homeostasis of rice seedlings under salt stress (Win et al., 2018).
18. Soaking seeds of some wheat, soybean, clover, and rice crop cultivars in *Nostoc* spp. and/or *Anabaena flosaquae* filtrates increased germination likely due to the hormones – abscisic acid, gibberellic acid and indole acetic acid and other metabolites (Abdel-Raouf, 2012).
19. *Plectonema, Nostoc, Calothrix, Scytonema, Hapalosiphon, Microchaete*, and *Westiellopsis* are microalgal species isolated from salt affected soils that may be effectively applied to ameliorate saline soils (Chatterjee et al., 2017; Lewis et al., 2019).
20. *Arthrospira* spp. may grow under pH and/or salt extremes (i.e. salt levels ranging from 7-15 g L^{-1}) (Uysal et al., 2015).

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