Chapter 23
Wastewater Cultivated Macroalgae as a Bio-resource in Agriculture

Maja Berden Zrimec, Erik Malta, Martha Bonnet Dunbar, Ana Cerar, Robert Reinhardt, and Rok Mihelič

Abbreviations

DW	Dry Weight
IMTA	Integrated-Multi-Trophic-Aquaculture
TN	Total Nitrogen

23.1 Introduction

Macroalgae and their extracts have a long tradition of being used in the coastal agriculture as the soil conditioners and enhancers of crop productivity (Nabti et al. 2016). Traditionally, seaweeds have been collected from the beach or harvested from the sea. The raising demand for their use for food (Shama et al. 2019) or interesting extracts (agar, alginate, carrageenin), however, resulted in their controlled production, mainly in the coastal seas and in lesser extent in the land-based systems.

Algae cultivation in the wastewater as the parallel (1) bioremediation and (2) biomass production presents an innovative industrial ecology model (Lawton et al. 2017). Nutrients, organic carbon and minerals that would otherwise be lost by the

M. B. Zrimec (✉) · A. Cerar · R. Reinhardt
Algen, Algal Technology Centre, LLC, Ljubljana, Slovenia
e-mail: maja@algen.si

E. Malta
CTAQUA, Aquaculture Technology Centre, El Puerto de Santa María (Cádiz), Spain

M. B. Dunbar
CTAQUA, Aquaculture Technology Centre, El Puerto de Santa María (Cádiz), Spain

ICMAN-CSIC, Institute of Marine Sciences of Andalusia, Spanish National Research Council, Puerto Real (Cádiz), Spain

R. Mihelič
University of Ljubljana, Biotechnical Faculty, Agricultural Department, Ljubljana, Slovenia

A. Ranga Rao, G. A. Ravishankar (eds.), *Sustainable Global Resources of Seaweeds Volume 1*, https://doi.org/10.1007/978-3-030-91955-9_23

discharge into environment, are recovered by algae for their growth. In wastewater cultivation, the large-scale production can be done without consuming large volumes of quality water and expensive commercial growth media. The produced biomass can't be used for human consumption because of the health regulations, except for algae grown in the Integrated Multi-Trophic Aquacultures (IMTA). It can still be exploited for a variety of products, from the low-added-value biofuels, organic fertilizers or biomaterials to the high-added-value compounds for pharmacy, cosmetics, and agriculture.

Macroalgae are an attractive opportunity for multiple industries because they can grow in various wastewaters and their production can be a source of additional jobs and income. In comparison to microalgae, they have an advantage of lower separation and dry mass preparation costs (Lawton et al. 2013; Ge and Champagne 2017). Harvesting is still one of the major setbacks for the large-scale microalgae production due to the costly separation of microscopic cells from the fairly dilute substrate, which usually requires expensive equipment and high energy consumption.

23.2 Wastewater Treatment with Macroalgae

The beginnings of algal cultivation in the wastewater can be traced back to the middle of the last century when W.J. Oswald in H.G. Gotass (1957) suggested that wastewater could be used for a large-scale algae production in the raceway ponds. Indeed, algae can recycle many chemical substances that could cause eutrophication or toxic effects if released into the environment. In the wastewater treatment plants, algal ponds can be used as a final polishing step in the third treatment stage or even as a combination of second and third stage due to the accompanying aerobic bacterial community.

Algae-bacteria community that establishes itself in the wastewater has a symbiotic relationship: algae produce oxygen that aerobic bacteria use for the degradation, while bacteria provide the nutrients and organic carbon for the algal growth. The produced oxygen considerably reduces costs of the energy-demanding technological oxygenation in the wastewater treatment process. Carbon dioxide is consumed in the photosynthesis, decreasing green-house-gas emissions. The odors are significantly reduced as well.

The removal efficiencies of nitrogen and phosphorus with macroalgae can reach levels higher than 90% (Neori et al. 1991; Mulbry et al. 2008; Ge and Champagne 2017; Ross 2017; Ge et al. 2018). The nutrient removal from the environment with macroalgae is most widespread in China where approximately 9,500 tons of phosphorus and 75,000 tons of nitrogen are removed annually by the coastal seaweed aquacultures (Xiao et al. 2017). Nevertheless, their large-scale cultivation still needs optimization. Wastewater is a highly variable medium and variations in the nutrient composition strongly influence the effectiveness of bioremediation.

Nutrient uptake depends most notably on the nitrogen form (NO_3^-, NO_2^-, NH_4^+, urea) and NO_3^-/NH_4^+ and N/P relative molar ratios that can limit the primary production. Ammonium is usually preferred source over the nitrate (Wallentinus 1984; Pedersen and Borum 1997; Abreu et al. 2011; Fan et al. 2014). At the initial NO_3^- and NH_4^+ concentration of 50 μM, *Graciliaria vermiculophylla* removed app. 40% of NO_3^- and 100% of NH_4^+ in just 4 hours (Abreu et al. 2011). Ammonium was removed preferentially also to urea, but the presence of urea enhanced uptake of other co-existing N-forms in the study by Ross (2017). Fan and co-workers (2014) observed that although *Ulva prolifera* preferred NH_4^+-N to NO_3^--N when the NO_3^--N/NH_4^+-N ratio was less than 2.2, the uptake of NO_3^--N was higher at the ratios between 2.2 and 12.9. N-uptake rate (33.9 ± 0.8 μmol·g^{-1} DW h^{-1}) was maximal at N/P ratio 7.5, while P-uptake rate (11.1 ± 4.7 μmol·g^{-1} DW h^{-1}) at N/P ratio 2.2 (Fan et al. 2014). NO_3^- uptake was faster than NH_4^+ at higher initial concentrations (450 μM NO_3^-, 150 μM NH_4^+) by *G. vermiculophylla* (Abreu et al. 2011).

Uptake efficiency was shown to be much higher at lower nutrient concentrations (Abreu et al. 2011). The nutrients' uptake rate can thus be substantially improved by adjusting the protocol of waste stream inflow dynamics, for example, by periodically applying lower nutrient concentrations. With step feeding *Chaetomorpha linum* with 10% centrate wastewater, Ge and Champagne (2017) increased nitrogen and phosphorus removal efficiencies from 72.3 ± 0.4% and 80.0 ± 0.3% to 86.8 ± 1.1% and 92.6 ± 0.2%, respectively.

The removal rates depend also on the algal species: the uptake of dissolved inorganic nitrogen from the aquaculture effluents by different species can vary as much as 16.9–96.6% (Ross 2017). Considerable research effort has therefore been put into the identification of most promising species for the wastewater treatment. The freshwater genera *Rhizoclonium*, *Cladophora* and *Oedogonium* (Cole et al. 2015, 2016b; Roberts et al. 2015a, b), and marine *Ulva, Cladocera, Gracilaria*, *Caulerpa* and *Sargassum* (Neori et al. 1991; Ross 2017; Arumugam et al. 2018) are few examples of the most promising macroalgae for bioremediation.

The performance of macroalgae in the wastewater is influenced by several biological factors, for example thallus morphology. Wallentinus and co-workers (1984) found that the species with filamentous, delicately branched, or monostromatic phenotypes had the highest rates of nutrient uptake because of the greater surface/volume ratio. These were short-lived, opportunistic algae like *Cladophora glomerata, Enteromorpha ahlneriana, Scytosiphon lomentaria, Dictyosiphon foeniculaceus* and *Ceramium tenuicorne*. Opportunistic macroalgae exhibit more rapid N uptake to maximally exploit the pulses of nutrient availability, while slower-growing, persistent species can have greater N-storage capacity (Pedersen and Borum 1997). The lowest uptake rates thus occurred among the late successional, long-lived, coarse species with a low surface/volume ratio (*Fucus vesiculosus, Furcellaria lumbricalls* and *Phyllophora truncata*). This might be especially relevant for the wastewater input regime and retention times. Uptake of nitrogen and growth rates can be higher following a period of N limitation in opportunistic species as algae strive to replenish their internal N pools ("surge uptake") (Pedersen and Borum 1997; Luo et al. 2012).

Macroalgae have the capacity to accumulate metals from the environment and can be employed in the wastewater treatment as a live (bioaccumulation) or dead (adsorption) biomass (Ross 2017; Michalak 2020). Good biosorption properties result from the macromolecules in the cell wall (e.g., polysaccharides, proteins) offering functional groups for binding metal ions (Michalak 2020). Biochar from macroalgae can also be used for metal and dye removal from the wastewater. These techniques can be used for the wastewater pre-treatment to enable more consistent and controlled influent for the biomass production.

23.2.1 Integrated Multi-trophic Aquaculture

Integrated Multi-Trophic Aquaculture (IMTA) is defined as »Enhanced production of aquatic organisms (with or without terrestrial organisms) of two or more functional groups, that are trophically connected by demonstrated nutrient flows and whose biomass is fully or partially removed by harvesting to facilitate ecological balance« (Dunbar et al. 2020). Primary producers play a key role as they "biofiltrate" inorganic nutrients from the waste of the primary culture, mitigating its impact on the environment and providing a potentially valuable crop. In general, seaweeds are favored over microalgae as the main primary producers of saltwater IMTA for the reasons already mentioned in the introduction (Chopin et al. 2001).

IMTA can be regarded as wastewater treatment integrated into aquaculture; however, it is far more than that. IMTA encompasses a more holistic, ecosystemic approach to aquaculture. Compared to other wastewater treatment systems, algae grown in IMTA are considered safe for human consumption as they are growing in effluent coming from the animal (usually fish) cultivation systems and not from systems containing human waste. It can be viewed as analogous to fertilization of terrestrial crops with manure.

The concept of IMTA has been known and practiced for centuries, albeit using different terminology until the first decade of this century (Neori et al. 2007). In many Asian countries, traditional practices show many examples of integrated aquaculture, such as the integration of carp culture in rice fields. In more recent years, seaweed and mollusk cultures in coastal areas have been integrated into the existing shrimp aquaculture (Edwards 2009; Soto 2009).

IMTA systems can be divided into two main groups. The first group is on-land IMTA, consisting of often compartmentalized systems that can be completely artificial (tanks) but more often comprise earthen ponds through which the water flow is led from the higher trophic level culture (e.g., fed fish) to the lower trophic levels (e.g., mollusks, seaweeds) (Fig. 23.1). The other group is at-sea IMTA (near-shore and off-shore), where the lower trophic level cultures are grown in proximity to and downstream from the higher trophic level cultures. Generally, algae associated with on-land cultures are relatively small, filamentous or foliose seaweeds, such as *Ulva, Codium, Gracilaria, Porphyra, Asparagopsis*; whereas seaweeds associated to at-sea cultivation are more often kelp species, such as *Saccharina latissima* (for instance with salmon farms; Chopin et al. 2001).

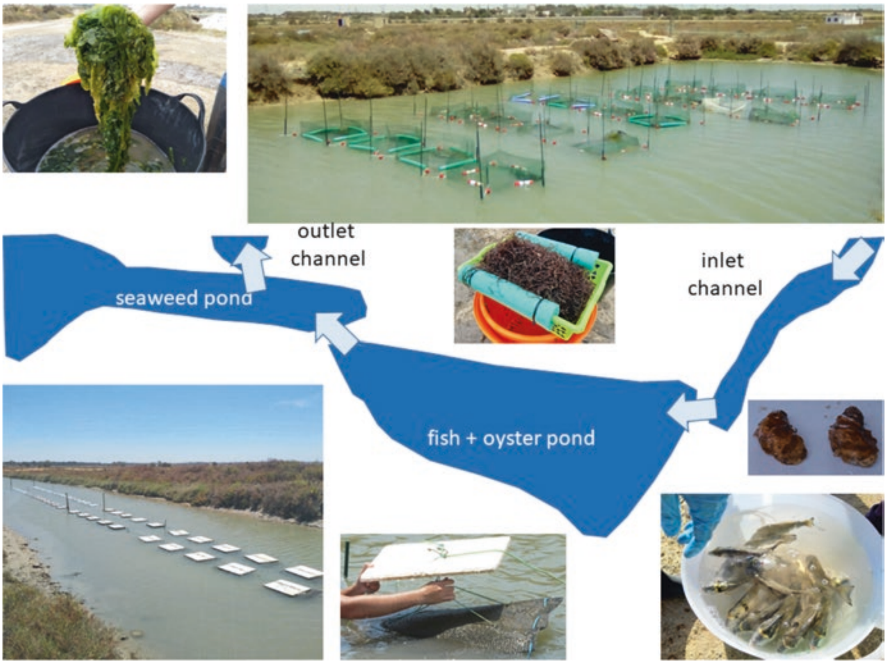

Fig. 23.1 Schematic representation of CTAQUA's pilot IMTA installation operating during the INTEGRATE project (2018–2019). Water flows are driven by tidal oscillations, arrows indicate direction. Fish (*Sparus aurata*, sea bream) and Pacific oysters (*Magallana gigas*) were grown in the same pond, which was hydrologically connected to the seaweed pond. Photos clockwise from top left: green seaweed *Ulva* sp., floating seaweed cages, red seaweed *Gracilaria gracilis*, Pacific oyster, sea bream, oyster bag, floating oyster bags. (Photos courtesy of CTAQUA)

23.3 Macroalgae Biomass production in Wastewater

The large-scale cultivation of macroalgae monocultures in the wastewater predominantly occurs in the open pond systems similar to the microalgae cultivation (Fig. 23.2): circular raceways with the paddlewheels for water circulation, maintaining the macroalgae in constant suspension (Lawton et al. 2017). The other predominant culturing system is algal turf scrubber (Fig. 23.3), which has a mixed algal community that is mostly self-seeded and uncontrolled (Mulbry et al. 2008; Lawton et al. 2017).

Wastewater promotes growth of a mixed culture with alternating strain composition, but for the bioproducts a more consistent material is needed. Some control over the culture composition can be provided by the wastewater pre-treatment, like biochar, filtration, dilution and macroalgae species with a tendency for dominant growth in a dynamic substrate, resistance to herbivory and infections, possibly exhibiting stable production rate and high nutrient uptake (Lawton et al. 2013; Ross 2017; Valero-Rodriguez et al. 2020).

Fig. 23.2 Wastewater treatment pond for macroalgae cultivation in Townsville, Australia. (Photo courtesy of Andrew J. Cole): *Oedogonium* sp. was cultivated in the treated municipal wastewater containing mean concentrations of approximately 4 mg· l^{-1} N and 0.8 mg·l^{-1} P (Cole et al. 2016a). Over a 12-months period, a tertiary treatment by *Oedogonium* reduced the concentrations of total N and P by 36% and 68%, resulting in 491 kg DW which recovered 24.4 kg N and 4.8 kg P. The algae were used as the feedstock to produce compost and biochar (Cole et al. 2016b)

The most commonly cultivated freshwater genera are currently *Rhizoclonium*, *Cladophora* and *Oedogonium*, the latest being most beneficial for the wastewater bioremediation and valorization (Cole et al. 2015, 2016b; Roberts et al. 2015a, 2015b). *Oedogonium* can have high biomass productivities (up to 35.7 g·$m^{-2}d^{-1}$ DW) when cultured in a variety of wastewater sources in Australia (Fig. 23.2) (Kidgell et al. 2014; Cole et al. 2015, 2016a; Lawton et al. 2017). The commonly researched seaweeds are from the genera *Gracillaria*, *Ulva* and *Sargassum* (Neori et al. 1991; Arumugam et al. 2018). Peak production for the seaweeds under optimal conditions can exceed 50 g DW $m^{-2}d^{-1}$ as was found for *Ulva lactuca* growing in the fishpond effluent in Eilat, Israel (Neori et al. 1991).

23.3.1 Physical Parameters

Light limitation is one of the main controllers of algal performance in the open systems, as the high concentrations of particulate matter in the wastewater effect the intensity of photosynthetically active radiation reaching the algae (Fig. 23.2). High

Fig. 23.3 First large-scale algal turf scrubber (ATS) system at the Patterson municipal wastewater treatment plant, California. (Photo courtesy Rupert J. Craggs). ATS improves water quality by passing a shallow stream of wastewater over the surface of a gently sloped flow-way (Craggs 2001). The system was 154.2 m long and 6.5 m wide, producing up to 62 g/m per day. Mat forming species were mainly cyanobacteria (mostly *Oscillatoria*), with canopy of filamentous algae (*Ulotrix* sp. and *Stigeoclonium* sp. prevailing in the summer) and diatoms (Craggs 2001)

concentrations of algae have a similar shading effect. This is why the inoculation density, culturing and harvesting regimes all contribute to the consistent biomass production. Lower inoculation density should result in a higher relative growth due to the higher availability of light and nutrients. In the study by Neori and co-workers (1991), the daily growth rates of *Ulva lactuca* ranged from 8.7% to 9.9% at a stocking density of 2 kg·m^{-2}, while they were considerably lower (0.6–4%) at higher densities. Favot and co-workers observed best specific growth rates and biomass production at *Ulva* sp. stocking density of 30 g·m^{-2} when studying a range between 15 and 60 g·m^{-2} (2019).

Light reaching the surface of the pond varies diurnally and seasonally. In the temperate regions, illumination periods are significantly shorter in winter, while summer months are critical due to the periods of high illumination causing photoinhibition and photodamage to the Photosystem II. A seasonal growth rates' variation of 1.78–6.23% was observed in *Graciliaria vermiculophylla* by Abreu and co-workers (2011). Temperature is another important environmental variable. Fan and co-workers (2014) found a considerable effect of temperature on the photosynthetic efficiency and N uptake rates in *Ulva prolifera*: in the temperature range of 5–30 °C, N uptake ranged from 177 to 543 mg·kg^{-1} DW h^{-1}.

The substrate depth in the pond is thus a balance among thermal stability, maximum illumination and photo-damaging effects. Although shallower ponds allow better light availability through the water column, the resulting temperature variation and photoinhibition could reverse the resulting positive effects.

23.3.2 Chemical Parameters

Growth rate and algal biomass composition highly depend on the wastewater chemistry. Ross (2017) demonstrated that various nutrient regimes, characteristic of the wastewaters, resulted in different daily growth rates, i.e., 4.75–11.2% in *Cladophora parriaudii* and 3.98–7.37% in *C. coelothrix*. The presence of urea in the medium enhanced growth and yielded a carbohydrate-rich biomass (38–54% DW) (Ross 2017). N/P relative molar ratios are important for the primary production although Liu and Vyverman (2015) found no marked change in the growth under eight different N/P ratios (ranging from 1 to 20): biomass productivities varied between 52.6 and 56.7 $mg{\cdot}L^{-1}d^{-1}$ DW in *Cladophora* sp. and 29.6–34.1 $mg{\cdot}L^{-1}d^{-1}$ DW in *Klebsormidium* sp.

Algal productivity depends also on the wastewater nutrients' loading rate. When growing a consortium of freshwater algae, dominated by *Microspora willeana*, *Ulothrix ozonata*, *Rhizoclonium hieroglyphicum* and *Oedogonium* sp. (Fig. 23.3), Mulbry and co-workers found that the mean algal productivity values increased from approximately 2.5 $g{\cdot}m^{-2}d^{-1}$ DW at the lowest loading rate (0.3 $g{\cdot}m^{-2}d^{-1}$ TN) to 25 $g{\cdot}m^{-2}d^{-1}$ DW at the highest loading rate (2.5 $g{\cdot}m^{-2}d^{-1}$ TN) (2008). Mean nutrient contents in the dried biomass increased 1.5–2-fold with increasing loading rate to a maximum of 7% N and 1% P (Mulbry et al. 2008). Step-feeding of *Chaetomorpha linum* with 10% centrate wastewater increased the biomass productivity by 26.5% compared to the single feeding of the total load (Ge and Champagne 2017).

pH and CO_2 application are significant as well. In *Oedogonium* cultures, maintained at a pH of 7.5 through the addition of CO_2, the biomass productivity was 8.33 ± 0.51 $g{\cdot}m^{-2}d^{-1}$ DW, which was 2.5 times higher than in control cultures not supplemented by CO_2 (3.37 ± 0.75 $g{\cdot}m^{-2}d^{-1}$ DW) (Cole et al. 2014). The rate of carbon fixation was 1380 $g{\cdot}m^{-2}$ $year^{-1}$ C and 1073.1 $g{\cdot}m^{-2}$ $year^{-1}$ C for cultures maintained at pH 7.5 and 8.5, respectively, and 481 $g{\cdot}m^{-2}$ $year^{-1}$ C for the control (Cole et al. 2014).

23.3.3 Culture Rotation

A year-round production is imperative for the industrial applications. A combination of species and culture rotation was proposed and successfully applied to mitigate the seasonal changes in light and temperature (Valero-Rodriguez et al. 2020). Valero-Rodriguez and co-workers found tropical *Oedogonium* sp. had highest specific growth rate in the summer conditions (36–40%), but the temperate *Stigeoclonium* sp. and *Hyalotheca* sp. had higher growth in the winter conditions (2020). When mixed, *Oedogonium* was dominant (>90%) in the warmer conditions, while *Stigeoclonium* and *Hyalotheca* prevailed in the colder ones. Their calculations suggest that a monoculture of *Oedogonium* and *Stigeoclonium* would produce 14.1 and 23.9 t·ha^{-1}year^{-1}, respectively, while a mixed culture would reach 24.4 t·ha^{-1}year^{-1}, showing a substantial improvement.

23.4 Macroalgae valorization for Agriculture

The utilization of algal biomass depends highly on its composition and active compounds. Both can be regulated by the cultivation conditions (Lawton et al. 2017; Ross 2017). Although wastewater's chemical composition is very variable, it can be to some point regulated by the pre-treatment or addition of inadequate chemicals. In any case, seaweed biomass cultivated in the wastewater should be examined for the multielemental composition before the further utilization (Michalak 2020). Potentially toxic elements are typical heavy metals such as As, Cd, Hg, Pb, but some of them are microelements necessary for the proper growth and development, e.g., Zn, Cu, Mn, Co, (Tuhy et al. 2014; Michalak 2020).

23.4.1 Biomass Composition

The biomass composition varies extensively among the species as found by Atkinson and Smith (1983) analysis of the C:N:P ratio in 92 macroalgae which varied from 183:9:1 to 3550:61:1. Smaller variations can be found also in the same species during different seasons or depending on the N source (Abreu et al. 2011; Ross 2017). The N and P content in biomass can also reflect the N/P ratio in the growth substrate (Liu and Vyverman 2015).

The most obvious difference arises in different N-regimes: macroalgae generally synthesize proteins and pigments when N is sufficient, and accumulate storage polysaccharides, such as starch, when they are under N-limitation (Smit et al. 1997; Cole et al. 2015). Freshwater macroalgae, growing in the nutrient replete media, have high rates of biomass production (often exceeding 15 g·m^{-2}d^{-1} DW) and nutrient uptake (Mulbry et al. 2008; Cole et al. 2015). At high productivities, 50–85% of the supplied nitrogen is incorporated into the algal biomass (Cole et al. 2015). Such algae may be suitable for the food and fertilizer, whereas N-starved algae may be better for the conversion into biofuels via digestion or fermentation processes (Ross 2017).

Macroalgae cultivated in the wastewater from animal production can provide a high-quality source of protein (Cole et al. 2015). *Oedogonium* biomass had an equivalent or higher protein quantity and quality than many terrestrial crops currently used as a source of protein in the animal feeds in Cole et al. study (2015). Additionally, *Oedogonium* accumulated calcium, potassium, magnesium and phosphorous.

23.4.2 Fertilizers

Macroalgae used as fertilizers can improve soil water-holding capacity, reduce erosion and nutrient leaching, increase soil organic matter, carbon, nitrogen, phosphorus and minerals, provide a substrate for the soil microbes, resulting in the increased growth and resilience of crops (Lawton et al. 2013; Sharma et al. 2014; Cole et al. 2015; Roberts et al. 2015a, 2015b; Nabti et al. 2016). They have an advantage of being biodegradable, non-toxic, non-polluting and non-hazardous to human, farm animals and birds (Tuhy et al. 2014; Nabti et al. 2016; Badescu et al. 2017).

Macroalgae cultivated in the wastewater are an effective slow-release fertilizer when applied as the untreated dried and milled biomass (Mulbry et al. 2008). Their effects on the plant mass and nutrient content can be equivalent to the effects of commercial fertilizers (Lawton et al. 2017) as they can have relatively high N and P biomass content. *Oedogonium intermedium* cultivated in the wastewater treatment plants, for example, recovered and concentrated in the biomass up to 5.4% N and 1.1% P (Cole et al. 2016a; Neveux et al. 2018).

Algal biomass with the recovered nutrients can be stabilized for the agricultural use by composting or pyrolysis (Cole et al. 2016b). Slow pyrolysis transforms the biomass into biochar. Biochar produced from *Oedogonium* biomass improved the retention of nutrients from fertilizer (N, P, Ca, Mg, K and Mo) in the low-quality soils and enhanced plant growth and the nutrient uptake (Roberts et al. 2015a, 2015b; Lawton et al. 2017). Radishes grown in the low quality, sandy loam soils with added biochar had 35–40% higher growth rates and 10–50% higher concentrations of the essential trace elements (Ca, Mg, K and Mo) and macronutrients compared to the radishes grown without biochar (Roberts et al. 2015a, 2015b; Lawton et al. 2017).

Algal biomass that was used as a biosorbent can be loaded with metal ions and thus an excellent addition to microelements-depleted soils (Tuhy et al. 2014; Badescu et al. 2017). Such biosorbents have additionally a high content of nitrogen and phosphorus and are readily biodegradable material with a high content of organic matter and other macronutrients (Ca, K) (Badescu et al. 2017). The bioavailability of metals is higher than in the traditional organic fertilizers (Tuhy et al. 2014). When *Ulva* sp. with bound Zn(II) ions (29.6 $mg \cdot g^{-1}$ of biomass) was used as a fertilizer, the content of zinc in the soil increased four-times in 8 weeks (Badescu et al. 2017). Similarly, Tuhy and co-workers prepared micronutrient fertilizer from the Baltic seaweeds and post-extraction residues, previously used as biosorbents of Zn(II) ions. Enriched biomass caused the biofortification of zinc and weight increase of garden cress (*Lepidium sativum*).

When the mature compost from the *Oedogonium intermedium*, cultivated in the municipal wastewater, was added to a low fertility soil, it significantly increased the production of sweet corn *(Zea mays*) (Cole et al. 2016b). Treatments receiving half nutrients with compost and half with mineral fertilizers as well as 100% compost treatment produced 4–9 times more corn biomass than when mineral fertilizer alone was added to the low fertility soil. Additional 15% corn productivity was achieved by addition of biochar, most likely due to its ability to bind labile N and P and prevent its loss from the soil (Cole et al. 2016b).

23.4.3 Bioactive Compounds

Plant biostimulants can be found in the macroalgae extracts and are any products that improve (a) nutrient use efficiency, (b) tolerance to abiotic stress, (c) quality traits or/and (d) availability of soil or rhizosphere confined nutrients (EU 2019). Additionally, several algae have been found to exhibit pesticidal activity (Nabti et al. 2016; Hamed et al. 2018). Bioactive compounds are usually obtained by the different methods of extraction and homogenization, which should be preceded by the biomass pre-treatments like washing to remove particles and impurities, drying, shredding, milling to get homogenous sample and sieving (Michalak and Chojnacka 2016). If enriched subfractions or purified preparations of seaweed extracts are used instead of the crude extracts, the problem of accumulation of salts or metals can be minimized (Nabti et al. 2016).

Macroalgae contain plant growth regulators including auxins, gibberellins and cytokinin, the latter being regarded as the most important in marine algae (Sharma et al. 2014; Michalak and Chojnacka 2016; Hamed et al. 2018). The extracts can promote shoot and root elongation, stimulate seed germination and root development, enhancement of frost and draught resistance, increased nutrient uptake and control of phytopathogenic fungi, bacteria, viruses, insects or other pests and restoration of the plant growth under high salinity stress (for more details see Sharma et al. 2014; Nabti et al. 2016; Hamed et al. 2018).

23.5 Conclusions and Future Perspectives

The on-land cultivation of macroalgae, especially in the wastewater, is still in its infancy. The scarce efforts nevertheless show a great promise. Macroalgae can easily grow in various wastewaters by recycling the nutrients and their productivity can be fairly impressive. Biomass composition is suitable for a range of products and can be further adjusted by the cultivation parameters.

Identifying the algae with best performance in a certain wastewater and environment is the first critical step in the wastewater cultivation, which usually starts with a laboratory screening and should be finally tested in the outdoor conditions, preferably over a whole annual cycle (Borowitzka 2013; Fort et al. 2019). First successful attempts of the annual macroalgae production in the seasonal climate areas have already been made by the culture rotation and utilization of the dominant species isolated from the local environment and should be further explored.

Using wastewater as the cultivating media is a sustainable way for nutrients' recycling and could pave a way to marketable prices. The issue is its very variable chemical composition. Although many strains of macroalgae can function well in such an environment, it is difficult to provide the biomass of constant quality and quantity, necessary for the marketable applications. Different pre-treatments can enable more controlled biomass composition and yield, including a multistep-algal-cultivation system where the biomass production ponds can be preceded by the wastewater treatment ponds.

The IMTA concept encompasses innovative and sustainable idea of utilizing trophically connected organisms to remove excess nutrients and waste from the aquatic environment by valorizing their biomass for different products. IMTA validity and great promise has already been thoroughly demonstrated. Its commercial implementation, especially in the western world, is nevertheless lagging. Improvements in the up-scaling of tested systems, stimulation of innovations in the methods of the different cultures, public outreach to improve general acceptance of aquaculture and a specific eco-label certifying the sustainability of IMTA are the main measures that could drive the industrial-scale adoption (Dunbar et al. 2020).

Macroalgae effectively uptake macro- and microelements from the wastewater, which makes them a rich-nutrition substrate for agricultural production or consumption, but such cultivation can also result in the biomass with toxic substances. Regular chemical analysis of the produced biomass is thus necessary to ensure a quality product. In the case of bioactive compounds, extraction and purification can eliminate the unwanted compounds.

For the algal system optimization, continuous monitoring of the parameters like oxidation-reduction potential, electric conductivity and oxygen concentration can enable optimal application of wastewater. Physiology measurements can be used to monitor algal performance, together with the nutrient concentrations, pH regulation with CO_2 etc. to fine-tune the bioremediation and biomass production.

More research is needed to further understand the roles of influence parameters. Recently developed kinetic models of algal and algal bacterial processes have high predictive power and provide deep insight into the actual processes. Model based control algorithms together with the information on environmental conditions enable significant increase in the productivity of algal ponds (Casagli et al. 2021) and can be used to further develop the large-scale high-production systems fed by the waste streams.

Although there is still a lot of work before the viable and marketable large-scale macroalgae cultivation in the wastewater is achieved, it is worthwhile goal to pursue. The same approach is already widely researched and developed with the microalgae, rapidly gaining in importance as it is supporting the care for our health and the environment.

Acknowledgements The Authors acknowledge projects: INTEGRATE - *Integrate Aquaculture: an eco-innovative solution to foster sustainability in the Atlantic Area*, funded by the ERDF through the INTERREG Atlantic Area 2014-2020 Programme (project grant number EAPA_232/2016); Water2Return - *Recovery and recycling of nutrients: turning waste water into added-value products for a circular economy in agriculture* (H2020 2017-2022) and LIFE AlgaeCan - Adding sustainability to the fruit and vegetable processing industry through solar-powered algal wastewater treatment (LIFE 16/ENV/EC 2017-2021).

References

Abreu MH, Pereira R, Buschmann A, Sousa-Pinto I, Yarish C (2011) Nitrogen uptake responses of *Gracilaria vermiculophylla* (Ohmi) Papenfuss under combined and single addition of nitrate and ammonium. J Exp Mar Biol Ecol 407:190–199

Arumugam N, Chelliapan S, Kamyab H, Thirugnana S, Othman N, Nasri NS (2018) Treatment of wastewater using seaweed: a review. Int J Environ Res Public Health 15:2851

Atkinson M, Smith S (1983) C: N: P ratios of benthic marine plants. Limnol Oceanogr 28:568–574

Badescu IS, Bulgariu D, Bulgariu L (2017) Alternative utilization of algal biomass (*Ulva* sp.) loaded with Zn(II) ions for improving of soil quality. J Appl Phycol 29:1069–1079

Borowitzka MA (2013) Species and strain selection. In: Borowitzka MA, Moheimani NR (eds) Algae for biofuels and energy. Developments in applied phycology, vol 5, pp 77–89. Springer Dordrecht Heidelberg New York London

Casagli F, Zuccaro G, Bernard O, Steyer JP, Ficara E (2021) ALBA: a comprehensive growth model to optimize algae-bacteria wastewater treatment in raceway ponds. Water Res 190:116734

Chopin T, Buschmann AH, Halling C, Troell M, Kautsky N, Neori A, Kraemer GP, Zertuche-González JA, Yarish C, Neefus C (2001) Integrating seaweeds into marine aquaculture systems: a key toward sustainability. J Phycol 37:975–986

Cole AJ, Mata L, Paul NA, de Nys R (2014) Using CO_2 to enhance carbon capture and biomass applications of freshwater macroalgae. GCB Bioenergy 6:637–645

Cole AJ, de Nys R, Paul NA (2015) Biorecovery of nutrient waste as protein in freshwater macroalgae. Algal Res 7:58–65

Cole AJ, Neveux N, Whelan A, Morton J, Vis M, de Nys R, Paul NA (2016a) Adding value to the treatment of municipal wastewater through the intensive production of freshwater macroalgae. Algal Res 20:100–109

Cole AJ, Paul NA, de Nys R, Roberts DA (2016b) Good for sewage treatment and good for agriculture: algal based compost and biochar. J Environ Manag 200:105–113

Craggs RJ (2001) Wastewater treatment by algal turf scrubbing. Water Sci Technol 44(11–12):427–433

Dunbar MB, Malta E-J, Agraso MM, Brunner L, Hughes A, Ratcliff J, Johnson M, Jacquemin B, Michel R, Cunha ME, Oliveira G, Ferreira H, Lesueur M, Lebris H, Luthringer R, Soler A, Edwards M, Pereira R, Abreu H (2020) Defining integrated multi-trophic aquaculture: a consensus. Aquac Europe 45(1):22–27

Edwards P (2009) Traditional Asian aquaculture. In: Burnell G, Allan G (eds) New technologies in aquaculture. Woodhead Publishing Series in Food Science, Technology and Nutrition, Woodhead Publishing, pp 1029–1063

EU Regulation 2019/1009 of the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products and amending Regulations (EC), No1069/2009 and (EC) No1107/2009 and repealing Regulation (EC) No 2003/2003

Fan X, Xu D, Wang Y, Zhang X, Cao S, Mou S, Ye N (2014) The effect of nutrient concentrations, nutrient ratios and temperature on photosynthesis and nutrient uptake by *Ulva prolifera*: implications for the explosion in green tides. J Appl Phycol 26:537–544

Favot G, Cunha ME, Quental-Ferreira H, Álvares Serrão ME (2019) Production of *Ulva* sp. in multitrophic aquaculture in earth ponds. Aquac Fish Stud 1(1):1–8
Fort A, Lebrault M, Allaire M, Esteves-Ferreira A, McHale M, Lopez F, Fariñas-Franco JM, Alseekh S, Fernie A, Sulpice R (2019) Extensive variations in diurnal growth patterns and metabolism amongst *Ulva* spp strains. Plant Physiol 180:109–123
Ge S, Champagne P (2017) Cultivation of the marine macroalgae *Chaetomorpha linum* in municipal wastewater for nutrient recovery and biomass production. Environ Sci Technol 51(6):3558–3566
Ge S, Madill M, Champagne P (2018) Use of freshwater macroalgae *Spirogyra* sp. for the treatment of municipal wastewaters and biomass production for biofuel applications. Biomass Bioenergy 111:213–223
Hamed SM, Abd El-Rhman AA, Abdel-Raouf N, Ibraheem IBM (2018) Role of marine macroalgae in plant protection & improvement for sustainable agriculture technology. Beni-Suef Univ J Basic Appl Sci 7:104–110
Kidgell JT, de Nys R, Hu Y, Paul NA, Roberts DA (2014) Bioremediation of a complex industrial effluent by biosorbents derived from freshwater macroalgae. PLoS One 9(6):e94706
Lawton RJ, de Nys R, Paul NA (2013) Selecting reliable and robust freshwater macroalgae for biomass applications. PLoS One 8(5):e64168
Lawton RJ, Cole AJ, Roberts DA, Paul NA, de Nys R (2017) The industrial ecology of freshwater macroalgae for biomass applications. Algal Res 24(Part B):486–491
Liu J, Vyverman W (2015) Differences in nutrient uptake capacity of the benthic filamentous algae *Cladophora* sp., *Klebsormidium* sp. and *Pseudanabaena* sp. under varying N/P conditions. Bioresour Technol 179:234–242
Luo MB, Liu F, Xu ZL (2012) Growth and nutrient uptake capacity of two co-occurring species, *Ulva prolifera* and *Ulva linza*. Aquat Bot 100:18–24
Michalak I (2020) The application of seaweeds in environmental biotechnology. In: Bourgougnon N (ed) Seaweeds around the world: state of art and perspectives. Advances in botanical research, vol 95. Elsevier Academic Press, Elsevier Ltd., 85–111
Michalak I, Chojnacka K (2016) The potential usefulness of a new generation of agro-products based on raw materials of biological origin. Acta Sci Pol Hortorum Cultus 15:97–120
Mulbry W, Kondrad S, Pizarro C, Kebede-Westhead E (2008) Treatment of dairy manure effluent using freshwater algae: algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. Bioresour Technol 99:8137–8142
Nabti E, Jha B, Hartmann A (2016) Impact of seaweeds on agricultural crop production as biofertilizer. Int J Environ Sci Technol 14:1119–1134
Neori A, Cohen I, Gordin H (1991) *Ulva lactuca* biofilters for marine fishpond effluents. II. Growth rate, yield and C: N ratio. Bot Mar 34:483–490
Neori A, Troell M, Chopin T, Yarish C, Critchley A, Buschmann AH (2007) The need for a balanced ecosystem approach to blue revolution aquaculture. Environment 49:37–43
Neveux N, Bolton JJ, Bruhn A, Roberts DA, Ras M (2018) The bioremediation potential of seaweeds: recycling nitrogen, phosphorus, and other waste products. In: La Barre S, Bates SS (eds) Blue biotechnology: production and use of marine molecules, vol 1. Wiley Online Library 217–239
Oswald WJ, Gotass HG (1957) Photosynthesis in sewage treatment. Trans Am Soc Civ Eng 122:73–105
Pedersen MF, Borum J (1997) Nutrient control of estuarine macroalgae: growth strategy and the balance between nitrogen requirements and uptake. Mar Ecol-Prog Ser 161:155–163
Roberts DA, Paul NA, Cole AJ, de Nys R (2015a) From waste water treatment to land management: conversion of aquatic biomass to biochar for soil amelioration and the fortification of crops with essential trace elements. J Environ Manag 157:60–68
Roberts DA, Paul NA, Dworjanyn SA, Bird MI, de Nys R (2015b) Biochar from commercially cultivated seaweed for soil amelioration. Sci Rep 5:9665

Ross ME (2017) Wastewater treatment by filamentous macro-algae. PhD, University of Edinburgh & SAMS

Shama A, Joyce SG, Mari FD, Ranga Rao A, Ravishankar GA, Hudaa N (2019) Macroalgae and microalgae: novel sources of functional food and feed. In: Ravishankar GA, Ranga Rao A (eds) Handbook of algal technologies and phytochemicals. Food, health and nutraceutical applications, vol I. CRC Press, USA 207–219

Sharma HSS, Fleming C, Selby C, Rao JR, Martin T (2014) Plant biostimulants: a review on the processing of macroalgae and use of extracts for crop management to reduce abiotic and biotic stresses. J Appl Phycol 26:465–490

Smit AJ, Robertson BL, du Preez DR (1997) Influence of ammonium-N pulse concentrations and frequency, tank condition and nitrogen starvation on growth rate and biochemical composition of *Gracilaria gracilis*. J Appl Phycol 8:473–481

Soto D (2009) Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper. No. 529. FAO, Rome 183

Tuhy L, Samoraj M, Michalak I, Chojnacka K (2014) The application of biosorption for production of micronutrient fertilizers based on waste biomass. Appl Biochem Biotechnol 174:1376–1392

Valero-Rodriguez JM, Swearer SE, Dempster T, de Nys R, Cole AJ (2020) Evaluating the performance of freshwater macroalgae in the bioremediation of nutrient-enriched water in temperate environments. J Appl Phycol 32:641–652

Wallentinus I (1984) Comparisons of nutrient uptake rates for Baltic macroalgae with different thallus morphologies. Mar Biol 80:215–225

Xiao X, Agusti S, Lin F, Li K, Pan Y, Yu Y, Zheng Y, Wu J, Duarte CM (2017) Nutrient removal from Chinese coastal waters by large-scale seaweed aquaculture. Sci Rep 7:46613