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Review

Integrated Marine Biogas: A Promising Approach towards Sustainability

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Abstract: Fossil fuel depletion, climate change, and increased global energy demands are the driving forces to find alternative sources of energy. Marine-based biorefinery has been recently discussed as a promising route to mitigate the environmental challenges, enhance the energy recovery, and provide a potential source for value-added products. Anaerobic digestion is a promising technology that can convert the organic compounds of marine ecosystems into biogas. To date, a comprehensive review incorporating integrated biogas potential and effective approaches to enhance seaweed digestibility for biogas production from marine resources has not been reported. Thus, the present review aims to explore and comprehensively present seaweed and other marine resources for potential biogas production. The basics and challenges of biogas production from seaweed are elucidated. The impact of biochemical composition on biogas and the microbial communities involved in anaerobic digestion of seaweed are discussed. Utilization of different techniques such as pretreatment, co-digestion, and sequential extraction of seaweed biomass to enhance the biogas yield and to mitigate the effect of inhibitors are presented. Specifically, this article evaluates the co-digestion of seaweed with other biomass feedstocks or liquid biowastes. Integration of marine microalgae cultivation on anaerobic digestate for value-added compound production, biogas upgrading, and bioenergy recovery provides a promising approach towards a zero-waste marine-based system.

Keywords: anaerobic microbes; seawater; seaweed; biogas; marine energy

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1. Introduction

In recent years, intensive studies have focused on the evaluation of rampant increases in emissions of greenhouse gases (GHGs) and the negative impacts of the global warming [1–3]. Man-made inputs have severely influenced the climate, with severe impacts on human health, the whole environment, and economic growth [4]. Currently, climate change is garnering much attention as it is posing serious threats. For example, it is currently reported that Europe's major rivers are shrinking under the most severe climate-driven drought in decades. The current situation puts humanity at a crossroads, motivating the global effort to achieve sustainable development goals. Energy consumption is a crucial factor for economic growth, which is one of the main causes of increased GHGs levels [5,6]. The estimated annual global energy consumption is 580 million terajoules, i.e., about 13,865 million tons of oil equivalents (mtoe) [7]. Since 2000, it has increased by about one-third and is expected to reach 740 million terajoules equivalent by 2040, representing a total increase of 77%. Therefore, the increased energy demand in the next 50 years cannot be met by the major oil exporters [8], which threatens the world economy and life on the planet. On the other hand, reliance on fossil fuel threatens the environment due to GHG emissions, which increase climate change [9]. Therefore, replacing fossil fuels with alternative clean and renewable energy sources is an utmost priority.

Solar, biomass, wind, geothermal, and hydropower are the most common renewable energy sources [10]. Renewable energy consumption increased by 3% in 2020, and bioenergy use also increased by 3% among many other renewable energy resources [11,12]. Bioenergy offers a promising alternative to fossil fuel. Biomass can be converted to bioenergy through two major technologies, namely thermochemical and biochemical processes [13,14]. Thermochemical processes mainly include pyrolysis, gasification, liquefaction, hydrothermal carbonization, and supercritical fluid extraction [15,16]. However, biochemical processes include alcoholic fermentation, anaerobic digestion, photobiological hydrogen production, and microbial fuel cells for energy production [14,16]. Many previous studies highlighted the pros and cons of each conversion method [17,18].

Currently, anaerobic digestion for biogas production is proposed as a promising renewable energy technology for mitigation of climate change by reducing the reliance on fossil-based fuels [19,20]. A wide range of wastes, including municipal and industrial wastewater, energy crops by-products, livestock waste, and food wastes, can be treated using this technology with triple purposes of energy recovery, soil improvement, and waste management. Compared to many other waste treatment processes, anaerobic digestion technology offers significant advantages (Figure 1). Biogas is the main product of this process as an energy product, while digestate is a by-product rich in nutrients and can be utilized as a soil fertilizer [21]. Among different biogas feedstocks, lignocellulosic biomass represents exciting characteristics for biofuels and other interesting products through a lignocellulosic biorefinery concept. It offers waste reuse, increases process efficiency, and reduces the environmental damage by integration of waste management, energy recovery, and valuable products generation [22,23]. Anaerobic digestion of lignocelluloses has received increasing attention in recent years. However, lignocellulosic biomass is composed mainly of hemicellulose, cellulose, and lignin, having many challenges for direct anaerobic digestion due to crystallinity, heterogeneity, and high polymerization degree of the biomass [24–26]. Efficiency of hemicellulose and cellulose de-polymerization to form sugars and lignin degradation are the main challenges for successful anaerobic digestion of lignocellulosic materials [27,28]. Therefore, biomass pretreatment could improve biodegradability and biomethane production of lignocellulosic biomass. It is essentially needed to remove lignin, expanding the fibers, and increase the accessible surface area of anaerobic microorganisms [28,29]. However, this step is energy intensive and highly costly, compromising the feasibility and sustainability of the process [30,31]. In addition to the aforementioned challenges, collateral flows such as excessive use of arable land, chemicals, or fresh water result in severe impacts on the environment [32]. Therefore, exploring new feedstocks to avoid competition for the available resources is of great importance. In this context, the estimated biomass productivity of seaweed is relatively higher (≈26 tons dry weight per hectare per year, compared to 2.3 tons for soya and 5.1 tons for corn). Seaweed also has high adsorption capacity that can be used for preservation of a healthy marine ecosystem and wastewater treatment through phycoremediation [28,33,34]. It can be used for anaerobic digestion, where seawater/wastewater is utilized instead of fresh water.

Recent studies discussed the marine biorefinery system through fermentation and bioethanol production [35,36], while there is a gap in the literature for evaluation of biogas production using marine resources. Therefore, intensive investigation for designing a feasible biorefinery approach for biogas production using marine resources is a timely topic. The present review article aims to provide an overview of the recent research on anaerobic digestion of marine biomass (seaweed) using seawater and marine microorganisms for biogas production. The strategies to enhance biomethane production from seaweed through liquid co-substrates, and the addition of external additives to the digestion system to promote the microbial communities are discussed. In addition, the potential of introducing marine microalgae to the system for achieving a zero-waste integrated approach is highlighted.

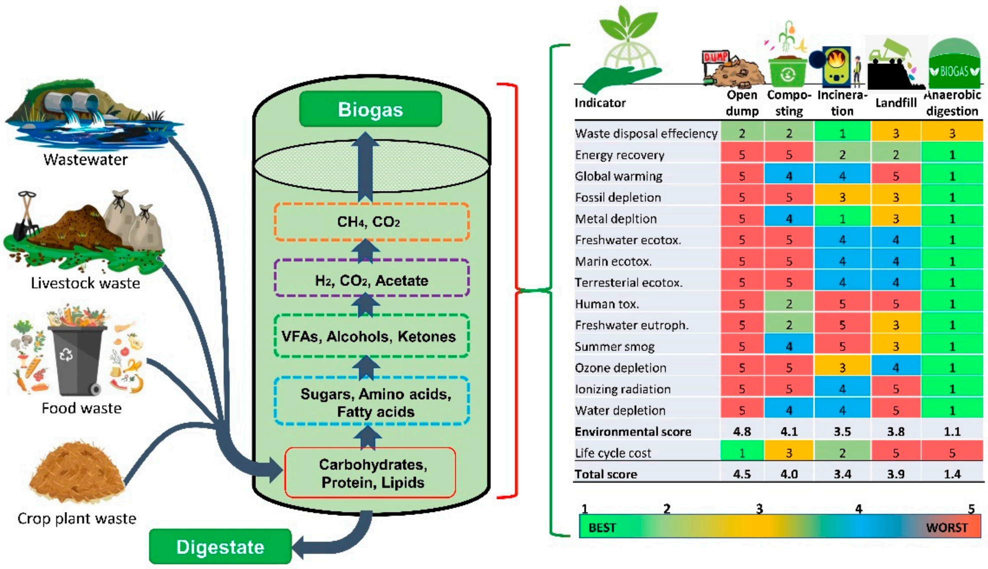

Figure 1. Comparing anaerobic digestion to other waste management routes using commonly used organic waste steams [28], copyright permission number 5345730119827.

A systematic search in several databases (ScienceDirect, Scopus, and PubMed) and free-access repositories (Google Scholar) was carried out based on the keywords "seaweed and marine energy" and "seaweed for biogas", and "anaerobic digestion of seaweed". This extensive information was further sub-classified into different sections as presented in this review, namely, anaerobic digestion (conventional and marine-based anaerobic digestion), potential of marine resources, availability, and co-digestion of seaweed. According to the ScienceDirect database (consulted in July 2022), 2034 articles with the keyword 'seaweed' in all kinds of energy have been published. Among such documents, 1466 research articles have been published, followed by 416 reviews, 36 short communications, 5 book reviews, and 2 conference papers. Further screening by keywords "seaweed anaerobic digestion" yielded a total of l553 articles of which 224 were research articles, 149 were review articles, 104 were book chapters, and the remainder were minor short communications and editorials. The studies used in the present review included all of those that discussed seaweed biomass conversion, specifically the integration of seaweed with other biomass such as microalgae. This clearly indicates the potential of seaweed for bioenergy production and the necessity to explore a marine-based biorefinery system for high efficiency.

2. Conventional Versus Marine-Based Anaerobic Digestion

2.1. Conventional Anaerobic Digestion

During anaerobic digestion, a number of synergistic reactions between microbial consortia degrade and convert organic feedstocks into biogas. The process includes four main phases, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 2), with specific microbial communities belonging to acidogenic, acetogenic, and methanogenic microbes. During the hydrolysis phase, high molecular weight organics (e.g., lipids, carbohydrates, and proteins) are first hydrolysed into smaller building units (i.e., fatty acids, glucose, and amino acids) by the action of acidogenic bacteria. The produced small molecules are further degraded by acidogenic bacteria into volatile fatty acids (VFAs) and other by-products such as CO_2, H_2S, and NH_3. VFAs are further digested during the acetogenesis phase by the action of acetogenic bacteria to produce H_2, CO_2, and acetate. Finally, methanogenic archaea utilize these intermediates during methanogenesis to produce biomethane and other gases [37].

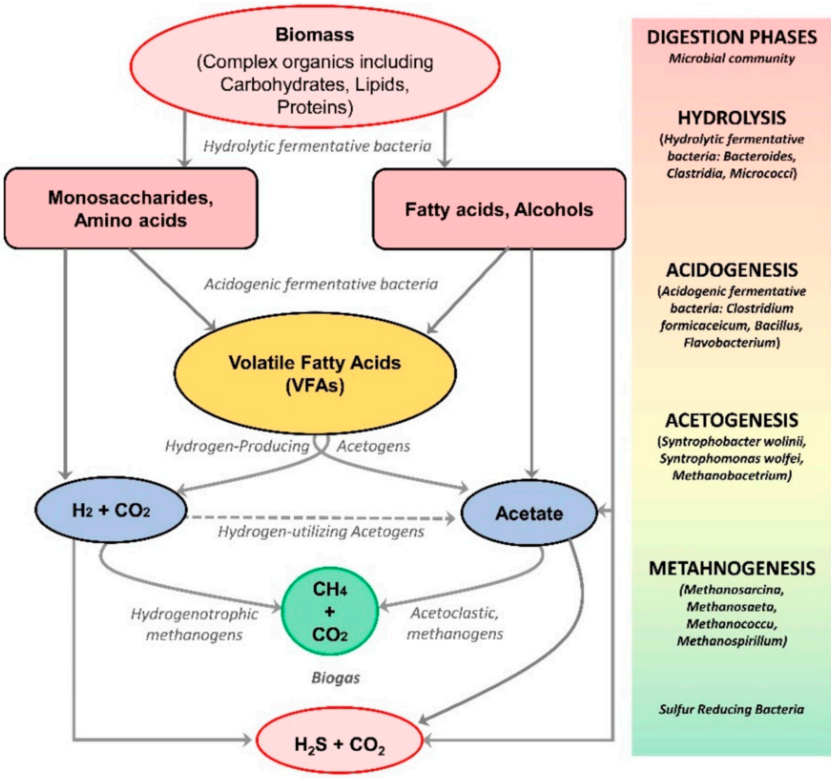

Figure 2. The four phases involved in anaerobic digestion of biomass showing the related microbial communities.

2.2. Marine-Based Anaerobic Digestion

Due to the shortage of fresh water in many countries, desalination technologies have received increasing attention. However, it is an energy-intensive process; energy consumption should either be reduced or compensated for with alternative renewable energy resources, such as wind power, solar energy, tidal power, geothermal energy, and anaerobic digestion coupled with desalination using plants/algae. Integration of these renewable energy technologies into desalination processes could significantly reduce GHG emissions [38]. Regarding anaerobic digestion as an energy source, the substitution of conventional fresh-water-based feedstocks with marine resources (seaweed and seawater) could be a promising approach for countries that lack adequate supplies of fresh water (Figure 3). In addition, it will significantly reduce the environmental impact due to the reduction of fossil fuel consumption. However, mono-digestion of seaweed still faces many challenges which result in low biogas yield. In addition, the digestate cannot be used directly as a biofertilizer and requires costly pretreatment to eliminate the heavy metals and residual salts.

Figure 3. Overview of anaerobic digestion using marine-based resources (seaweed and seawater).

3. Potential of Marine Resources for Biofuel Production

Marine environment, with the depth reaching beyond 10 km, covers more than 70% of the Earth's surface, and it is the natural habitat for over one billion microbial species and thousands of macroscopic species [39,40]. It is estimated that all marine organisms make up approximately 50% of the global biodiversity. These marine organisms have diverse biochemical compositions and can be used for outstanding bioactivities and nutraceutical enriching profiles due to their excellent adaption to a wide-range of variations in the marine environment. More than 30,000 marine-derived bioactive compounds described so far have been used as valuable sources of food bioactive ingredients, nutraceuticals, cosmeceuticals, pharmaceuticals, and many other applications [39]. In recent years, photoautotrophic organisms have been discussed as a promising candidate for energy sustainable production, with more interest dedicated to algal biomass as third-generation biofuel feedstocks [20,38,41]. They are more advantageous due to the elevated cost of lignin degradation for conversion of second-generation biofuel feedstocks, such as lignocellulosic wastes [42,43], and competition of first-generation edible feedstocks with the available resources [44]. Therefore, R&D for algae-based biofuels in the last decade has grown considerably to counter these issues. Specifically, marine microalgae [45,46] and seaweed [47,48] have been discussed as potential feedstocks for integrated biofuel production, seawater desalination, and heavy metal removal. In that context, net energy of 11.0 GJ ton^{-1} of dry weight (dw) can be generated from seaweed, compared to 9.5 GJ ton^{-1} dw from microalgae, both of which are much higher than that of terrestrial plants. The highlights of review articles published and a comparison with current review articles are summarized in Table 1.

Table 1. Key points of some previous published papers on marine resources for biorefinery, compared to current review articles.

Feedstocks	Study Highlights	References
Seaweeds	• Marine macroalgal waste valorisation is reviewed considering a biorefinery approach. • A world perspective concerning marine macroalgal waste was provided. • Agricultural and feed applications were reviewed aiming at the direct use of the biomass. • Cascade valorisation routes were presented considering high- and low value-products. • Valorisation routes were extensively discussed focusing on future perspectives.	[49]

Table 1. *Cont.*

Feedstocks	Study Highlights	References
Genetically modified algae	• Focused on biosafety concerns and regulatory frameworks of Fourth Generation Biofuel (FGB). • Discussed genome-scale engineering to boost biomass production. • Highlighted the current and prospective challenges of FGB production. • Extracted whole-genome sequences as a basic prerequisite for molecular analysis. • Strong national and international commitments were needed to develop FGB.	[50]
Seaweeds	• Methods of pre-treating algal biomass for utilization were summarized and compared. • Bioconversion of algal biomass into value-added products was reviewed. • Strategies for the improved bioavailability of algal biomass were suggested. • Challenges and trends for future development of algal biorefinery were discussed.	[51]
Algae and shellfish waste	• Review on thermal co-processing of lignocellulosic biomass, algae, and shellfish waste. • Design, operation, and key parameters of pilot-scale reactors were discussed. • Techno-economic analysis and synergistic effects of co-processing were discussed. • Co-pyrolysis and co-gasification for high energy recovery and high profitability. • Carbon credit makes thermal co-processing more sustainable to utilise biomass.	[52]
Seafood waste	• Seafood production was associated with the generation of large amounts of waste biomass responsible for huge nutritional losses. • The waste biomass can be used as a source of valuable nutrients and also other industrial compounds including bioenergy. • Green processing can help eco-friendly utilization of the waste. • Algal technology and biorefinery can recover the compounds on a commercial scale. • Green processing helps improve blue economy and achieve sustainable development.	[53]
Seaweeds	• Seaweed-based regenerative ocean farming can efficiently restore marine ecosystems. • Seaweed was discussed as a good source of protein and bioactive compounds for human and animal consumption. • Seaweed showed the potential to be a future source of energy and renewable plastics. • Seaweed offers coastal nations a viable source of revenue and a bioeconomy option.	[54]

Table 1. *Cont.*

Feedstocks	Study Highlights	References
Marine resources (seaweed, seawater, and marine microorganisms)	• The potential of marine resources (seaweed, seawater, and marine microbes) for energy production are discussed in detail. • A promising technology that can convert organic compounds of marine resources into biogas are discussed. • A comprehensive review encompassing integrated biogas potential and effective approaches to enhance seaweed digestibility for biogas production are highlighted. • Utilization of different techniques such as pre-treatment, co-digestion, and sequential extraction of seaweed biomass to enhance the biogas yield and to mitigate the effect of inhibitors are presented. • Integration of marine microalgae cultivation through anaerobic digestate recovery for value-added compounds production, biogas upgrading, and bioenergy generation is discussed as a promising approach towards zero-waste.	Current study

3.1. Natural Resources of Marine Biorefinery

A closed-loop marine route using seawater, seaweed, halophilic microalgae, and an adapted microbial community could be a potential combined system for efficient biofuel production through a zero-waste biorefinery approach. In that context, the measurable goals of economic, social, and environmental benefits including profitability, renewability, water conservation, carbon neutrality, and minimum waste generation are the key end points when developing a sustainable biorefinery platform. Biorefinery is a technology that translates biomass into multiple products through a cascading sustainable process. Sustainability metrics for marine biomass are measured as the percentage of valuable product recovery, maximum energy and water efficiency, zero-waste generation, and optimal return on investment. Marine biorefineries aim to boost the establishment of a circular bio-economy as a more efficient resource management of bio-based renewable resources by transiting from the current linear economy to a sustainable circular one [55]. Seaweed biomass can be obtained primarily from naturally-grown seaweed by wild harvesting or through aquaculture-based production in seaweed farming. Comparatively, wild harvesting is limited and cannot meet the growing industry demand [39]. Therefore, a marine-based biorefinery system for seaweed farming could be a practical solution towards sustainable climate mitigation as well as water, energy, and food security.

3.2. Seawater

Seawater represents about 97% of the global water and covers about 71% (i.e., 3.6×10^8 km^2) of the earth's surface [40,56,57]. It is considered as a renewable water source and is readily accessible in almost all countries including those suffering freshwater shortages. Energy conversion/production using seawater is a highly desirable approach. Many inorganic compounds such as $NaCl$, $MgSO_4$, $MgCl_2$, $CaSO_4$, $MgBr_2$, K_2SO_4, and K_2CO_3 are present in natural seawater. Amongst these, NaCl is the major constituent for seawater salinity, representing about 3.5% (55% chloride and 30% sodium) of seawater [58]. The salinity of seawater is influenced by the season of the year and the climate of the region. For instance, water salinity in the Red Sea and the Mediterranean Sea can reach up to 4.1% and 3.9%, respectively, which is much higher than in Wonthaggi and Australia where it is 2.8% [59,60]. No matter the level of salinity, water and minerals required for the growth of microbial communities during anaerobic digestion can be sustainably provided by seawater.

Several technologies/methods have been applied to generate energy from seawater, such as hydrogen production by photocatalysis, photoelectrochemical methods, and biochemical methods. Due to the presence of essential ions such as chloride, sodium, sulfate, and magnesium (Table 2), seawater can act as a catalyst in the biomass fractionation and as an alternative source of nutrients in the conversion processes [61–63].

Table 2. Average chemical composition of seawater. Data modified from Ref. [58].

Element	Concentration Range (g/L)
Cl	19.5–22.0
Na	10.8–14.0
Mg	1.3–1.5
S	0.9–3.2
Ca	0.37–0.42
K	0.38–0.46
Br	0.07
C	0.03
N	0.01

Seawater-Mediated Biofuel Production

Seawater contains a lot of natural inorganic and organic matter that can be used indirectly to produce biofuel through microbial or seaweed cultivation. Microorganisms such as phototrophic bacteria, cyanophytes, and dark-acidogenic bacteria can grow in seawater, producing H_2 and/or VFAs [64–67]. Furthermore, phototrophic bacteria have the potential to degrade VFAs for H_2 production [68]. Specific microorganisms trigger fermentation reactions in the presence of nutrients at slightly alkaline pH [68]. Other operating conditions such as light intensity, temperature, characteristics of marine bacterial strains, and availability of nutrients are crucial factors that influence the efficiency of H_2 production. Among them, optimization of temperature and pH are the main influencing factors that can significantly enhance H_2 production efficiency through nutrient supplementation in seawater [68]. Microalgae can be grown in seawater, and biomethane can be generated by anaerobic digestion of microalgal biomasses in a seawater medium. However, it might result in microbial inhibition due to the high concentration of Na^+ ions which are toxic to the microbial communities. Hence, marine sediments have been suggested as a source for salt-tolerant anaerobic microbes for CH_4 production using microalgae and seawater [69,70]. In that context, Miura et al. evaluated [71] various marine sediments as promising microbial sources for biomethane fermentation of the phaeophyte *Saccharina japonica* at seawater salinity and observed that all studied marine sediments were able to produce VFAs, while one of the studied sediments showed enhanced biomethane production due to complete conversion of the produced VFAs. It was attributed to dominance of acetoclastic methanogens belonging to the *Methanosarcina* genus after cultivation. Thus, establishing anaerobic microbial communities for marine-based anaerobic digestion can be achieved.

Various studies reported successful anaerobic digestion of seaweed (Table 3), where the wet marine biomass contains approximately 3% ash with similar salinity as seawater [72]. Marquez at al. [73] examined three microbial inocula, namely marine sediment, cow manure, and sea wrack-associated microflora for biogas production. Among them, marine sediment was recommended as the best source for microbial communities for anaerobic digestion of sea wrack biomass with seawater, where the average biomethane produced was 94.33 mL g^{-1} VS. This finding confirms the possibility of biomethane production even if seawater is added as liquid substrate when appropriate microbial communities are used. Other studies reported marine sediment as an active site of biomethane production, where marine sediments from sublittoral and littoral locations were evaluated for biomethane production from *Macrocystis pyrifera* in a seawater medium. Littoral sediment showed higher activity of methanogenesis with enhanced biomethane yield of 217.1 mL/g VS, which was comparable to that reported using a freshwater medium. All of these studies shed light on the importance of energy resources of seashore and island communities. However, the biomethane yield of the marine anaerobic digestion system is still much lower than the theoretical values [71]. Since different microbial communities have great differences due to different marine habitats [74], it is speculated that marine sediment of different ecosystems might have significant impact on biomethane production potential from seaweed.

Table 3. Summary of some previous studies that reported anaerobic digestion of seaweed.

Seaweeds	Mode	Remarks	Refs.
Cladophora sp. and *Ulva intestinalis*	Batch/ Continuous	• Batch tests of 100 mL were assessed by co-digesting *Cladophora* sp. and *Ulva intestinalis* from the Gulf of Riga with wheat straw and straw pellet. • Mono-digestion showed the lowest BMP for *Ulva intestinalis* (277.7 mL CH_4/gVS) and the highest for the *Cladophora* sp. 523.3 CH_4/g VS. • Co-digestion resulted in a slight increase in the synergy index (i.e., from 1.9% to 4.7%) but not for all co-digestion trials.	[75]
Ulva rigida + sugar industry wastewater	Batch/ Continuous	• The optimal inoculum for biogas production was obtained from mixing decomposed macroalgae with diluted anaerobic sludge from a wastewater treatment plant. • A SMY of 76 mL/g VS and a biomethane content of 75% were obtained from continuous co-digestion of dried and powdered *Ulva rigida* and sugar industry wastewater at a weight ratio of 50:50 performed in an anaerobic up-flow reactor. • PCR-DGGE results suggested a significant change occurred in the bacterial and archaeal communities during a four-month continuous anaerobic digestion.	[76]
Laminaria digitata/ *Saccharina latissima* with dairy slurry	Batch/ Continuous	• Higher specific biomethane yield (SMY) was obtained during batch digestion tests by acclimatized inoculum obtained from continuous macroalgae digesters compared to inoculum obtained from digesters treating grease trap waste and slurry. • Continuous co-digestion of natural L. digitata and dairy slurry operated more efficiently with higher L. digitata fraction (67% of VS) at an organic loading rate (OLR) of 5 g VS/L/d, generating a SMY of 232 mL/g VS at an MC of 57%. • Continuous co-digestion of cultivated *S. latissima* and dairy slurry achieved a higher SMY of 252 mL/g VS at *S. latissima* fraction of 67% (VS) but lower OLR of 4 g VS/L/d. • Continuous mono-digestion of both macroalgae were affected at the OLR not exceeding 4 g VS/L/d.	[77]

Table 3. *Cont.*

Seaweeds	Mode	Remarks	Refs.
Green pea + *Laminaria digitata*	Continuous	• Biomethane production from digestion of green peas was inhibited by excessive VFAs, when only 2% of substrate was replaced with *L. digitata* at an OLR of 2.67 g VS/L/d. • Certain macroalgae constituents even at trace concentrations were more inhibitory to the traditional methanogens than to the other microbes such as acidogens. • Effective microbe adaption and start-up strategies, including initial very low macroalgae addition at a low OLR, can reduce the inhibitory effect whilst enhancing the process stability; consequently, co-digestion was stable at an OLR of 1.25 g VS/L/d comprising 35% of macroalgae, generating a biogas yield of ca. 500 mL/g VS at MCs of 55–65%.	[78]
Ulva sp. + sewage sludge	Batch	• An optimal SMY of 139 mL/g co-substrate was achieved using dehydrated *Ulva* sp. and sewage sludge at a dry weight ratio of 6:94. • Further increase of algae fraction resulted in a sharp decline in biogas production. • A TS reduction of 59% and a VS reduction of 75% were achieved.	[79]
Macrocystis pyrifera, *Durvillea Antarctica*/their blend	Anaerobic sequencing batch reactor (ASBR)	• Both algae species have similar biogas productions of 180.4 mL/g dry algae per day, with a biomethane concentration around 65%. • The same methane content was observed in biogas yield of algae blend; however, a lower biogas yield was obtained.	[80]
Ulva rigida	Batch	• Through mixing decomposed macroalgae with anaerobic sludge and water, yielding into 408 mL of biogas. • The process was then investigated in a sequencing batch reactor (SBR) which led to an overall biogas production of 375 mL with 40% of biomethane. • A high biogas production yield of 114 mL/g VS added was obtained with 75% of methane.	[77]

3.3. Marine Microalgae

Many recent studies focused on the synthesis of useful compounds by marine microorganisms for various purposes including functional biochemical production, animal feed, and human food, as well as energy recovery. Among these microorganisms, halophilic and halotolerant microalgae have been recognized as promising organisms for biotechnology research owing to their diversity, CO_2 fixation, and high biomass productivity. So far, many eukaryotic and prokaryotic (Cyanophyta) microalgae are identified as promising candidates for value-added products and biofuel, and some are currently available on an industrial scale [81]. Halophilic microalgae include a wide variety of salt tolerant organisms, which are not only resistant to high salinity but can adsorb and concentrate the solutes in their cells at higher concentrations than the surrounding environment [82]. For instance, the green microalga *Dunaliella salina* is a common halophile with the ability to live at high salt concertation of 3 M NaCl [83], and it showed the highest growth of 2.32 g L^{-1} at salinity of 150% through stepwise adaptation. Microalgae from saline inland and estuaries zones, from brackish marsh to high salt environments, can be isolated and used as salt tolerant species with great potential for biomass and biochemical production as well as seawater bio-desalination [84].

It was reported that the halophyte *Pheridia tenuis* sequesters salt in the vacuoles with the potential to obtain salt-free water after cultivation [85]. Many algal species, including *Spirulina* sp. and *D. salina*, can produce β-carotene, glycerol, lipids, and essential omega-fatty acids that can be used as food supplement sources [86,87]. Khazraee et al. [88] utilized effluents of a dairy wastewater treatment plant as substrate in the anode, while *Chlorella vulgaris* was inoculated in the cathode. The salinity removal was 0.341 g^{-1} day^{-1} with higher growth of 38%. The synergistic effect of the microalga *Scenedesmus abundans* with bacteria was investigated using the organic compounds present in effluent of a petroleum refinery. Maximum cellular density of 2.058 and chlorophyll and carotenoid concentrations of 2.78 and 1.365 g mL^{-1}, respectively, were recorded. Interestingly, some freshwater microalgae such as *Chlorella vulgaris* and *Scenedesmus* sp. showed high chloride ion removal efficiency with enhanced lipid accumulation at high salinity, which provides a feasible technology for dual application in seawater desalination and biodiesel production [89]. However, the biodesalination concept is a new topic, and related research is still in its infancy that could be useful for integrated marine biogas production.

3.4. Seaweed

The fast growth of seaweed and other marine plants results in the formation of a thick canopy layer that affects the growth of suspended phytoplanktons due to the inhibition of light penetration. Thus, the rate of photosynthesis decreases, resulting in a reduction of dissolved oxygen required for aquatic organisms. In addition, the accumulation of seaweed at the coastal areas poses many aesthetic and environmental issues. Generally, millions of tons of naturally-grown seaweed are washed up on coastal areas as a waste, which should be removed and disposed of by dumping in landfills to maintain eco-labels such as Blue Flag Beach category for tourism [90]. However, landfills have many issues and negative environmental impact [91]. Recently, seaweed has been discussed as an alternative and renewable energy source [38,92]. Seaweed comprises mainly three groups belonging to red, green, and brown algae [93] that have been discussed for the production of various biofuels [20]. Global seaweed production has been rising rapidly in recent years, reaching 32.4 million tons in 2018 with an average increase rate of 10% over 10 years [94]. Out of the total seaweed production, seaweed farming accounts for 96.7%, whereas wild harvesting accounted for 3.3% in 2015 [94]. The biochemical composition of seaweed biomass varies considerably based on the species and growth conditions, composed mainly of carbohydrates, proteins, and lipids (Table 4). Compared with lignocellulosic biomass, seaweed has the potential of high yields with no requirements of intensive fertilization or arable land [95]. Seaweed requires only seawater, CO_2, sunlight, and inorganic nutrients to grow, making it a promising feedstock for biomass and energy production [96]. Additionally, it is devoid of lignin which enhances biomass biodegradability compared to lignocellulosic biomass [97]. In addition to the commercial possibilities of seaweed farming, it has a high potential of CO_2 fixation and nutrient adsorption, subsequently lessening the eutrophication of water and enhancing sustainable biomass supply [98].

Table 4. The biochemical composition of representative brown, red, and green seaweed.

Seaweeds	Carbohydrates (%)	Lipids (%)	Proteins (%)	References
Brown				
Dictyopteris australis	33.1	9.7	1.3	[99]
Laminaria digitata	46.6	1.0	12.9	[100]
Saccharina japonica	51.0	1.0	8.0	[101]
Undaria pinnatifida	43.0	4.0	24.0	[102]
Stoechospermum marginatum	33.6	10.9	3.9	[99]
Red				
Gracilaria vermiculophylla	34.5	0.24	35.3	[103]
Gracilaria gracilis	28.6	1.7	13.7	[104]
Acanthophora spicifera	11.6–13.2	10.0–12.0	12–13.2	[105]
Palmaria palmata	39.4	3.3	22.9	[100]
Hypnea valentiae	11.8–13	9.6–11.6	11.8–12.6	[106]
Green				
Ulva reticulate	33.3	2.5	6.9	[105]
Cladophora glomerata	34.7	2.4	13.7	[104]
Ulva rigida	15.8	1.5	13.7	[77]
Codium decorticatum	50.6	9	6.1	[99]
Halimeda macroloba	32.6	9.9	5.4	[107]

3.4.1. Biofuel Production from Seaweed

Among several biomass feedstocks, renewable biofuel generation from seaweed has gained much attention in recent years due to many advantages over first- and second-generation biofuel feedstocks [108]. The appearance of naturally-grown marine macroalgae waste (MMW) at the coastal regions is a regular phenomenon influenced by eutrophication, wind, temperature, and wave episodes [109,110]. There are several reports about the negative impacts of MMW accumulation all over the world [111,112]. Seaweed waste lacks the appropriate management as it is mostly sent to landfills or left unmanaged, representing a loss of renewable resources and ultimately leading to coastal degradation, health problems, environmental consequences, and economic issues by affecting the tourism and beach-based commerce [109,110]. Therefore, seaweed has been discussed as an attractive feedstock for bioenergy and other value-added products [109,113,114].

Adoption of macroalgal biomass via thermal conversion for bio-oil, anaerobic digestion for biogas production, and fermentation for ethanol (Figure 4) could provide a promising solution for marine pollution [115]. Among thermal conversion methods, direct combustion, pyrolysis, and gasification require a dry feedstock, where the drying step has a significant negative impact on the energy balance and the return on investment. In that context, seaweed water content (80%–90%) which is generally much higher than many terrestrial plants (sugarcane~75%, and grain maize 14%–31%), makes it a less feasible source for pyrolysis [116–118]. However, hydrothermal conversion could provide a feasible route for bio-oil production from wet macroalgal biomass. More detailed discussion on the pros and cons of thermochemical conversion of seaweed has been published recently [41]. In addition, recycling of anaerobic digestate sludge to the HTL system could enhance the energy recovery due to higher organic load (Figure 4). Similarly, the residual biomass from the fermentation process can be efficiently converted to biogas, which was reported to enhance energy recovery [47]. For anaerobic digestion, biodegradation of recalcitrant compounds occurs during hydrolysis phase, which is the rate-limiting phase for the entire process [119]. Low cellulose content and absence of lignin [109], together with the presence of easily fermentable carbohydrates, increase the potential of seaweed for biogas production [120]. Although seaweed has a preferable cellular structure to terrestrial plants, successful biodegradation is still a fundamental issue for solubilisation of recalcitrant compounds and efficient degradation of organic macromolecules into simple compounds.

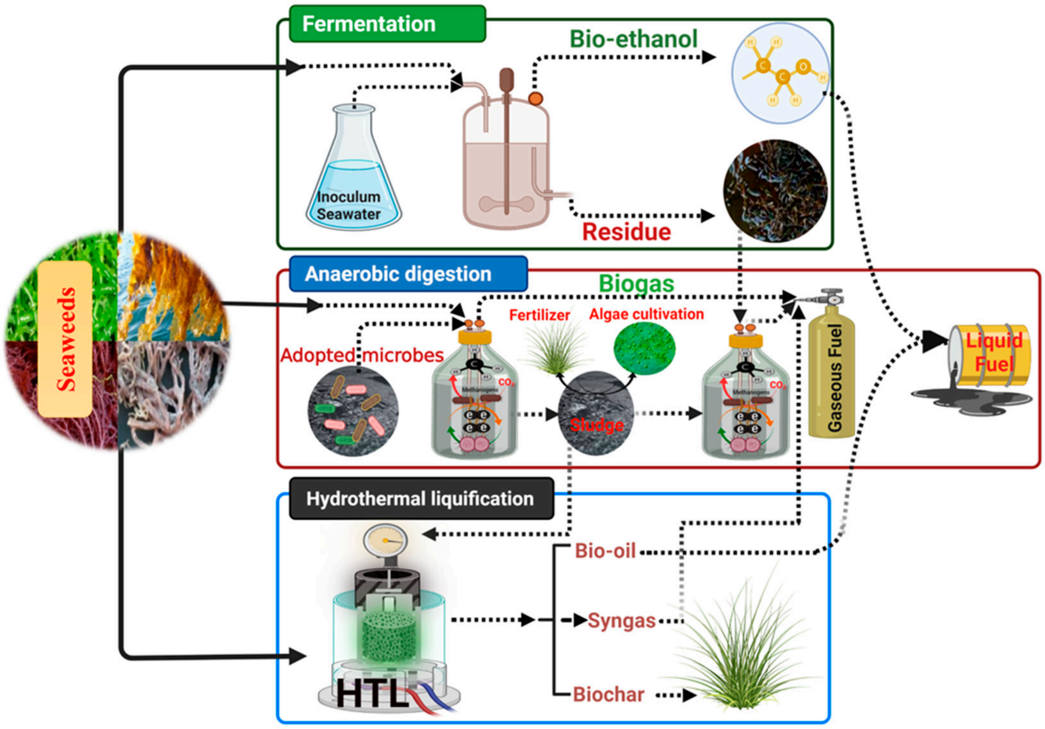

Figure 4. Different routes of biofuel production from seaweed for enhanced energy recovery and zero-waste approach.

3.4.2. Anaerobic Digestion of Seaweed

Anaerobic digestion of MMW, mainly composed of the phaeophyte *Saccorhiza polyschides*, showed the maximum biogas yield of 227 mL/g VS in 53 operational days and maximum biomethane content of 64.5% in 51 days due to the increase of total solid content up to 2.5% [121]. At the end of anaerobic digestion, 43% COD reduction and 46% VS reduction were recorded. Many other studies reported the high efficiency of seaweed for biogas production (Table 5). It was reported that anaerobic digestion of seaweed can produce up to 447.8 mL/g VS of biomethane without any pretreatment. Compared to terrestrial plants and microalgae, the highest recorded biomethane yield was 334 mL/g VS and 284–287 mL/g VS, respectively (Table 5). These results indicate the potential of seaweed as a feasible feedstock for biogas production.

Sequential energy recovery is another aspect that has been discussed recently to enhance the efficiency of biomass conversion (Figure 5). The strategy of sequential or co-production of biofuels could enable the complete utilization of seaweed biomass for enhanced biofuel production. Co-production of biofuels is carried out based on biochemical composition in which biofuels are produced sequentially. For instance, carbohydrate-rich seaweed could be used for sequential biohydrogen and biomethane production, collectively called hythane. The potential of *Ulva reticulata* for hythane production was studied to increase the disintegration potential through chemo-mechanical pretreatment. The pretreatment enhanced the biomass disintegration, induced liquefaction at a minimum specific energy of 437.1 kJ/kg TS, and resulted in higher hydrogen production of about 63 mL H_2/g COD [122]. In addition, *S. latissima* was evaluated for consequent biohythane generation and showed an improved solubilization trend with enhanced biomethane production to 345.1 mL/g VS, and the maximum energy conversion efficacy of 72.8% was achieved after two-stage biohythane production [123].

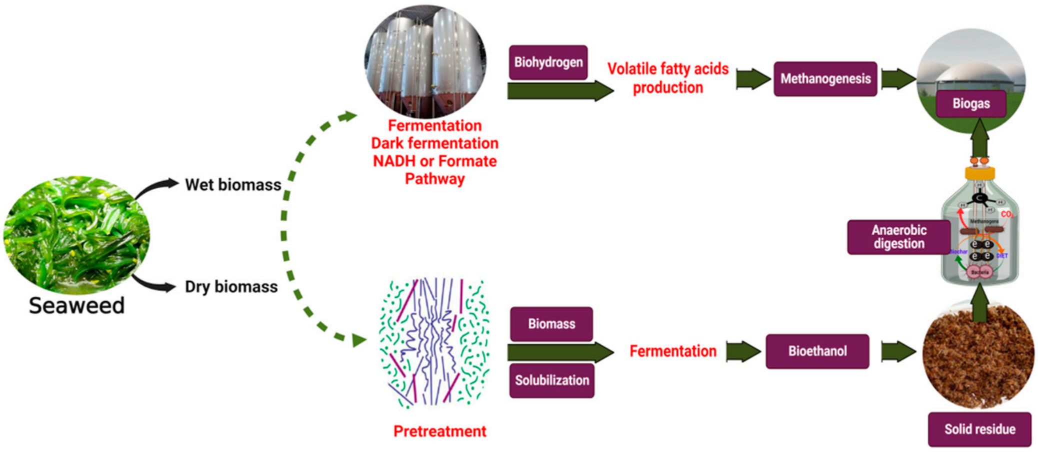

Figure 5. The principal of sequential energy recovery from seaweed.

Sequential product recovery could also enhance the process of energy recovery from seaweed. In that context, sequential utilization of two *Laminaria* sp. for phytoremediation, recovery of value-added products, and bioenergy recovery was suggested [124,125]. Value-added products (including pharmaceutical polysaccharides and protein) were initially extracted; then residual biomass was used for anaerobic digestion, which showed higher biomethane production of 523 and 535 mL CH_4/g VS for *L. digitata* and *Laminaria saccharina*, respectively. In addition, anaerobic digestion performance of the whole and agar-extracted *Gracilaria multipartita* biomass was compared [126]. Results showed that biogas yield was enhanced after agar extraction, which was attributed to lipid and long chain fatty acids reduction in the residual biomass. Moreover, seaweed anaerobic digestion has much shorter T_{80} compared to that of lignocelluloses. For instance, T_{80} of *Gracilaria multipartita* was 12 days [126], while it was 15 days for rice straw [127]. Lignocelluloses pretreatment can reduce T_{80}, where it was reduced from 21 days for anaerobic digestion of raw rice straw to 14 days after pretreatment with anaerobic digestate [128]. Interestingly, agar extraction prior to anaerobic digestion of seaweed was reported to reduce T_{80} from 12 days to 11 days [126], which is much better compared to lignocelluloses. From an economic aspect, agar pre-extraction from *Gracilaria multipartita* biomass enhanced the annual revenue to USD 36,087 per ton^{-1}, compared to USD 1253 per ton^{-1} for the whole seaweed biomass [126]. Therefore, sequential anaerobic digestion and establishing integrated approaches could enhance the economy of the whole process. During anaerobic digestion, the microbial community is the main indicator for successful reactor performance as discussed in the next section. Thus, understanding the microbial community distribution and dynamics could help researchers better predict and establish successful marine-based anaerobic digestion systems.

Table 5. Comparison of pre-treated microalgae, lignocellulosic biomass, and unpretreated seaweed biomethane potential.

Biomass	CH_4 Production (mL/g VS)	References
Microalgae		
Phaeodactylum tricornutum	284–287	[129]
Nannochloropis salina	247	[130]
Rhizoclonium	145	[131]
Phormidium sp.	223	[132]
Nannochloropis salina	233	[130]

Table 5. *Cont.*

Biomass	CH_4 Production (mL/g VS)	References
Lignocellulosic biomass		
Wheat straw	295 299 285 334	[133]
Rice straw	207 261 203	
Maize stalk	267 254 272	
Seaweeds		
Sargassum sp.	260–380	[134]
Saccharina latissima	340	[135]
Laminaria digitata	232	[76]
Saccharina latissima	252	[76]
Laminaria japonica	350	[136]
Pelvetia canaliculata	386	[137]
Laminaria hyperborea	280	[138]
Sargassum sp.	92.18	[139]
Ulva intestinalis	447.8	[75]
Laminaria digitata	327	[140]

3.5. Microbial Communities in a Marine-Based Biogas System

The processes involved in anaerobic digestion including application of pretreatment can significantly influence the microbial communities and abundance, which ultimately affect the biogas production. Azizi et al. [141] studied the changes in microbial communities under both mesophilic and thermophilic anaerobic digestion of the Rhodophyte *Gracilaria* sp. The abundant bacterial orders included *Clostridiales*, *Synergistales*, and *Bacteriodales*; while *Coprothermobacter* sp. showed dominance under thermophilic conditions due to its proteolytic activity at high temperatures (50–70 °C). However, archaeal communities showed an abundance of *Methanobacteriales* and *Methanomassiliicoccales* under thermophilic and mesophilic conditions, respectively, with a highest cumulative biomethane yield under a thermophilic environment (246.1 mL CH_4/g VS). In addition, Sun et al. [142] evaluated the microbial performance during anaerobic digestion of seaweed and attributed the obtained sequences into five major groups *Firmicutes*, *Bacteroidetes*, *Synergistales*, *Spirochaetes*, and *Proteobacteria*. The prevalence of phylum *Bacteroidetes* during anaerobic digestion of seaweed was correlated to the high protein content. Additionally, increased OLR provides a higher protein-input to the digestion system, which results in *Bacteroidetes* enhancement. With the increase of substrate, *Bacteroidetes* bred rapidly, leading to an accumulation of VFAs in the system. Order *Methanobacteriales* was the major methanogen among the detected archaea in the reactor. When OLR are elevated, a mass of substrate is fermented and converted to H_2/CO_2 by bacterial communities, which is beneficial to the *Methanobacteriales* growth. Enrichment of *Methanobacteriales* is related to the hydrogenotrophic methanogens pathway for biomethane production from microalgal biomass [143]. In that context, Jung et al. [144] stated that *Methanotrichaceae* was the major methanogenic group, indicating that aceticlastic methanogenesis was likely the major pathway for biomethane production using sulfur-rich seaweed biomass. Hydrogenotrophic methanogens, particularly *Methanomicrobiales*, became more abundant by increasing the OLR in a mesophilic reactor. These results suggest the contribution of hydrogenotrophic methanogenesis to biomethane production at higher OLRs. In contrast to *Methanomicrobiales*, the *Methanobacteriales* proportion decreased as the OLRs increased, which can be attributed to the higher sensitivity of

Methanobacteriales to H_2S toxicity and organic overload than *Methanomicrobiales* [145,146]. Thus, *Methanomassiliicoccales*, *Clostridiales*, *Methanobacteriales*, and *Synergistales* are the main microbial communities that should be enriched during microbial adaptation for application in marine-based anaerobic digestion. However, different profiles of pH, VFAs, and feedstock composition significantly alter the microbial communities and are required to be studied per case.

4. Challenges Associated with Marine Biogas Production

Anaerobic digestion of seaweed is still in the infancy stage as it is studied mostly in labs. Despite various advantages of seaweed over other biofuel feedstocks, there are some challenges that need to be overcome in order to reach industrial-scale levels [147]. Seaweed anaerobic digestion has major bottlenecks due to the elevated content of high molecular weight organic compounds and relative rigidity of the cell wall, which hinders the hydrolysis process [148]. In addition, seaweed biomass contains several inhibitory compounds such as sulfate that hampers the biomethanation and causes subsequent microbial inhibitory effects. High sulfur content results in the generation of hydrogen sulfide during anaerobic digestion by sulfate-reducing bacteria, leading to competition between methanogens and sulfate-reducing bacteria for acetate, and subsequently lesser biomethane yield [149]. Some seaweed has low biodegradability index, e.g., *Ascophyllum nodosum* and *Fucus serratus* (0.19–0.34, respectively, when anaerobically digested for 30 days), which results in low VS degradation where 66–81% of VS cannot be degraded [123]. In addition, availability of carbohydrates in seaweed differs by seasonal variations, geographic locations, and sometimes the carbohydrate content is relatively lower than the acceptable level for efficient biogas production [150].

Seaweed has the advantage of excess cations, such as sodium, potassium, and calcium, which were reported to enhance the microbial communities. For instance, Jard et al. [113] investigated the effect of K^+ and Na^+ on anaerobic digestion and found that both have significant impact on the digestion process. However, results showed that K^+ has more impact on biomethane production, which was attributed to the relatively higher initial content of Na^+ (3.1 g L^{-1}) in the inoculum than K^+ (0.4 g L^{-1}). Thus, the evaluation of cations content in the feedstock, seawater, and inoculum is of great importance to design a successful anaerobic digestion process in seaweed. Heavy metals represent another concern during anaerobic digestion, where high concentrations may be inhibitory to the microbial population. At optimum concentration, heavy metals play a significant positive role for enhanced anaerobic digestion [151]. However, Nkemka et al. [152] investigated the impact of heavy metals in mixed seaweed of the Baltic Sea on anaerobic digestion and reported that a high concentration of cadmium poses a significant negative impact during anaerobic digestion. However, higher biomethane yield was achieved using iminodiacetic acid (IDA) cryogel which can remove heavy metals from seaweed hydrolysate before anaerobic digestion.

For decades, seaweed has been discussed as a potential natural source of antimicrobial products that may help curb antibiotic resistance in livestock [153,154]. Various studies attributed the antimicrobial and other biological activities of seaweed to polyphenolic compounds [153,155]. Despite the advantageous characteristics of polyphenolics in seaweed as bioactive compounds, they have an inhibitory effect on anaerobic digestion [148]. It is reported that the higher the polyphenol levels present, the lower the level of biomethane produced is. Thus, initial extraction of polyphenols and using the residual biomass for anaerobic digestion could enhance the economy of the whole process. Another limiting factor for anaerobic digestion of seaweed is ammonia inhibition due to high protein content which results in a low C/N ratio. The reported optimal C/N ratio for anaerobic digestion ranges from 20 to 30. At lower ratios, nitrogen will be released and accumulate in the form of ammonia ($NH_4{}^+$). In addition, high nitrogen contents were reported to inhibit methanogens, which result in the accumulation of VFAs that leads to failure of the process [98]. Thus, excessive high $NH_4{}^+$ concentrations increase the pH value leading to a toxic effect on the microbial communities. In addition, unionized ammonia leads to proton imbalance, while ionized ammonia inhibits the enzymes incorporated in biomethane production [98]. Overall, the composition of seaweed and seawater complicates the evaluation of the substrate as a feed source for anaerobic digestion, which can be mitigated using different strategies as discussed in the following section.

The challenges related to implementing seaweed biorefinery include techno-economic feasibility and efficient biomass conversion to a viable output. Recent studies stated that energy production coupled with bioremediation using seaweed biomass for combined bioethanol and biogas production can render the energetic sustainability using energy and transportation. However, high production cost, low productivity, and cost-intensive downstream processing have been the major bottlenecks in developing large-scale systems [156,157]. A recent study on the economic feasibility of *Sargassum* sp. biomass in anaerobic digestion estimated an annual gross biomethane production of $3.02 \times 10^5/m^3$. The annual gross energy yield and profit were 1083.9 GJ and USD 68,738, respectively [112]. Although the study showed promising results, the production cost of biomethane from seaweed is economically unfeasible yet unless the production of valuable by-products (such as agar, alginates, mannitol, and iodine) can be considered [158]. In terms of pollution abatement, anaerobic digestion of seaweed is a valuable technology [148], while it requires future efforts, especially from the engineering and industrial point of view. These efforts would make the seaweed biomass economically competitive and allow the integration of different production systems to reduce the process cost and achieve a zero-waste approach [159].

5. Strategies to Boost Marine Biogas Production

As discussed in the previous section, anaerobic microbial activity is adversely affected by various inhibitors such as sulphide, ammonia, polyphenols, and heavy metals, [160]. To address the challenges associated with CH_4 productivity, some technologies have been explored aiming at OM bioavailability for microbial hydrolysis, thereby reducing the hydraulic retention time (HRT) and enhancing biogas generation [159]. Various pretreatment methods, including physical, thermal, chemical, and biological methods, have been applied to enhance seaweed digestibility (Table 6). The pretreatment aims to degrade the cell wall architecture, improve the hydrolysis of seaweed polymers, and overcome undesired compound formation that might inhibit the subsequent microbial metabolic activity. Despite alkali and acid pretreatment showing effective action in swelling fibers and hydrolyzing polymers, it increases the risk of inhibitory compound generation and consequently appears less attractive than other chemical treatments, such as peroxides and enzymes. Washing seaweed in fresh water was suggested to eliminate the inhibitory salts, but the value of this pretreatment warrants further research to reduce the impacts on sustainability due to the requirement of fresh water. On the other hand, seaweed accumulates metal ions by its negatively-charged polysaccharide components, which not only reduces the biogas yield but requires further evaluation to ensure the safety of the digestate for further applications.

In summary, it is possible to design a suitable seaweed biogas production system with proper pretreatment that could maximize its sustainability for biofuel production at the lowest cost. A possibility to increase the extent and rate of biodegradability during seaweed digestion is to use adapted inoculum to the high salinity and specific anaerobic digestion conditions of seaweed. In addition, ammonia inhibition can be controlled by adjusting the C/N ratio through co-digestion with other substrates containing a high C/N ratio [161]. In addition, the low organic nutrient content of seawater can be compensated for by the addition of wastewater (liquid co-digestion). The inhibitory effect due to high content of heavy metals or indigenous bioactive compounds such as polyphenols can be mitigated by sequential processing of biomass through initial extraction followed by anaerobic digestion of residual biomass.

Table 6. Biogas/biomethane enhancement of seaweed by various pretreatments.

Pretreatment	Feedstock	Pretreatment Conditions	AD Process	HRT (d)	Incubation Temp. (°C)	Results	Change in Energy Potential (%)	Refs.
				Physical				
Mechanical	Laminariaceae	Beating; 580 rpm; 10 min	Batch	21	50	430 mL CH_4/g^{TS}	+53	[120]
	P. canaliculata	Beating; 580 rpm; 60 min	Batch	21	37	340 mL CH_4/g^{VS}	+74	[108]
	P. canaliculata	Beating; 580 rpm; 10 min	Batch	21	37	444 mL biogas/g^{TS}	+179	[162]
	F. serratus					181 mL biogas/g^{TS}	+183	
	F. vesiculosus					231 mL biogas/g^{TS}	+220	
	L. digitata					157 mL biogas/g^{TS}	+52	
Microwave	*Laminaria* sp.	50 Hz; 560 W; 30 s	Batch	38	25	244 mL CH_4/g^{VS}	−26	[163]
				Biological				
Bm-2 strain white rot fungi and Trametes hirsuta,	*Mexican Caribbean macroalgae Consortia*	35 °C; 6 d	Batch	29	35	104 mL CH_4/g^{VS}	+20	[164]
Enzymatic broth	*L. digitata*	40 °C; 24 h	Batch	32	35	86 mL CH_4/g^{VS}	−6	[165]
Cellulase		37 °C; 24 h				225 mL biogas/g^{VS}	−1	
				Chemical				
Acids	*L. digitata* 2.5% citric acid	120 °C; 1 h; 1 atm	Batch	32	35	237 mL biogas/g^{VS}	+4	[165]
	1% lactic acid					161 mL biogas/g^{VS}	−42	
	6% lactic acid					101 mL bio-gas/g VS	−226	
	6% oxalic acid					83 mL bioga/g VS	−275	
	6% citric acid					69 mL bioga/g VS	−330	
				Thermal				
Autoclaving	*Sargassum* sp.	121 °C; 1 bar; 30 min	Batch	42	37	541 mL CH_4/g^{VS}	+60	[166]
Steam explosion	*S. latissima*	130 °C; 10 min 160 °C; 10 min	Batch	119	37	268 260	+20 +17	[167]

5.1. Anaerobic Co-Digestion

5.1.1. Biomass Co-Digestion

Anaerobic co-digestion (AcoD) refers to digestion of at least two substrates which have complementary properties to achieve better performance and to provide better nutrient balance with enhanced biogas production [168]. The major benefits of co-digestion include enhanced system stability and biomethane yield through synergistic effects which promote more diverse microbial communities, better nutrient balance (especially C/N ratio and trace elements), and dilution of toxic compounds including heavy metals [169]. The typical C/N ratio of macroalgae is generally as low as 14 due to high protein content [28], which varies widely based on the species, location, and season. Thus, it is necessary to add co-substrates rich in carbon to balance the nutrient supply and dilute the inhibitory compounds, which ultimately ensures a stable digestion process. For instance, seaweed biomass was recently suggested as a co-substrate for anaerobic digestion with lipidic-rich waste, which could achieve the desired C/N ratio and nutrient balance [28]. Anaerobic co-digestion also has another benefit on the economical scale because it provides the possibility of sharing facilities and equipment, which reduces the total investment cost [28]. The co-digestion synergistic impact on biogas production takes place due to interaction between different substrates, which is correlated to the supplementation of micronutrients and trace elements or any other parameter that ultimately influences digestibility of the substrate [170,171]. For instance, a synergistic effect was recorded during co-digestion of *Laminaria japonica* with sewage sludge or food waste at different mixing ratios. The study showed that increasing the seaweed proportion up to 75% with food waste significantly increased the biomethane yield, which was attributed to the relatively higher sulphur content in the seaweed biomass. Thus, the content of trace metals in the seaweed biomass plays a significant role in co-digestion, which provides advantageous characteristics with metal-deficient wastes [136]. Tabassum et al. [140] investigated mono- and co-digestion of brown macroalgae, where co-digestion of *L. digitata* with dairy slurry at a VS ratio of 67:33 showed high anaerobic digestion efficiency at a relatively high OLR of 5 g VS L^{-1} d^{-1}, which resulted in SMY of 232 mL/g VS. In addition, co-digestion of *S. latissima* with dairy slurry achieved a higher SMY of 252 mL/g VS at a lower OLR of 4 g VS L^{-1} d^{-1} [76]. In the same context, co-digestion of both fresh and dried *Ulva* sp. with cattle slurry showed up to 17% more biomethane yield than mono-digestion [172].

5.1.2. Wastewater Co-Digestion

Water-borne waste, which contains either OM and/or essential inorganic nutrients that promote microbial activity, represents a potential hazard to natural water systems [173]. Therefore, wastewater mixed with seawater can be used to cultivate the macroalgae [41] or co-digested directly with seaweed for enhanced anaerobic digestion efficiency. In that regard, Tabassum et al. [77] achieved a stable digestion process when *Ulva rigida* was co-digested with sugar industry wastewater at a weight ratio of 50:50. In addition, co-digestion of seaweed (15%) with waste activated sludge (WAS, 85%) was feasible with 26% higher biomethane production than WAS alone without decreasing the overall biodegradability of the substrate (42–45% biomethane yield) [174]. Some liquid byproducts also can be used to be co-digested with seaweed. For instance, glycerol is one of the byproducts produced from the biodiesel industry, and nowadays, its production exceeds the commercial demand [103]. Options for biological conversion of glycerol into valuable products are becoming increasingly important, including anaerobic co-digestion with several substrates [103]. Glycerol is an easily acidifying compound rich in carbon, while its amendment as a co-substrate should be carefully evaluated in order to prevent reverse process imbalance. In general, the addition of glycerol up to 6% was reported to significantly boost the biomethane production, but higher concentrations resulted in an inhibitory effect [175,176]. However, Oliveira et al. [177] assessed the optimal conditions for biomethane production from the macroalga *Sargassum* sp. co-digested with glycerol and waste frying oil. Results showed that the biochemical biomethane potential of *Sargassum* sp. was 181 L CH_4 kg^{-1} COD, while co-digestion with glycerol and waste frying oil increased the biomethane potential (BMP) by 56% and 46%, respectively. Co-digestion of glycerol, seaweed (*G. vermiculophylla*), and sewage sludge was evaluated, where the addition of 2% glycerol (*w:w*) increased the BMP by 18%, achieving almost complete methanation of the substrate ($96 \pm 3\%$) with specific biomethane production of 599 L CH_4 kg^{-1} VS [104]. However, an inhibitory effect was recorded with the addition of 5% glycerol. A significant increase in the specific biomethane production was also observed by co-digestion of the seaweed *G. vermiculophylla* with sewage sludge (605 L CH_4 kg^{-1} VS) compared to mono-digestion.

Co-digestion of Batik wastewater with dried marine seaweed *Gracillaria verrucosa* (BW:DG-50:50) showed a significant increase in cumulative biogas and biomethane production, with average values of 23.18 mL (biogas) and 11.59 mL (biomethane), compared to 13.83 mL and 6.92 mL, respectively, for inoculum and 11.82 mL and 5.92 mL using fresh biomass [178]. In another example for wastewater application, the feasibility of co-digestion of *Ulva* sp. with whey was investigated at varying substrate mixing ratios which confirmed the beneficial effect of whey on biomethanation of *Ulva* sp., with a biomethane yield up to

1.6-fold higher than mono-digestion of *Ulva* sp. [179]. Overall, liquid anaerobic co-digestion of seaweed with marine water mixed with wastewater is still not definitive, but it is worthy of further investigation and optimization.

5.2. Integrated Marine Biorefinery

The phenomenon of use and recover, reduce, re-use (U&3Rs) endeavors to create a zero-waste strategy for a supply chain via comprehensively restoring as well as regenerating resources in the industrial and natural ecosphere [180]. In practice, it endeavors to produce zero waste through system-wide innovations to recover value from what was traditionally called "waste". Zero-waste practices pertain to novel strategies for reducing resource usage and recovering value from the common waste flow [181]. For example, the residues of seaweed after extraction of value-added compounds such as lipids, proteins, carbohydrates, and agar may be used for bioenergy production through anaerobic digestion, creating an alternative source of renewable energy. Thus, the overarching objectives of the current review can be extended to the zero-waste strategy, and the potential of possible outcomes outlined above can provide additional downstream products from marine biogas systems (Figure 6). In many studies, seaweed was selected based mainly on its potential to be used for biofuel production [76,112,182], and the digestate of seaweed might be restricted to being used as a fertilizer due to the high content of heavy metals [183]. In addition, other studies focused on using seaweed to remove heavy metals [112], which makes the digestate worse for further application as a biofertilizer. Although a biofuel-based zero-waste strategy prioritized biogas yield, digestate can be used as a potential growth medium for marine microalgae cultivation. The produced biogas can be used for microalgae cultivation where CO_2 is utilized by microalgae, and CH_4 is upgraded. Introducing value-added compound extraction and other routes for energy recovery from microalgal biomass will have a significant positive impact on the whole system efficiency through the suggested integrated zero-waste approach. In addition to the suggested approach, artificial intelligence has been recently integrated in different processes including biogas production processes [184], which could be inevitable for further enhanced biogas recovery from seaweed in a marine-based system.

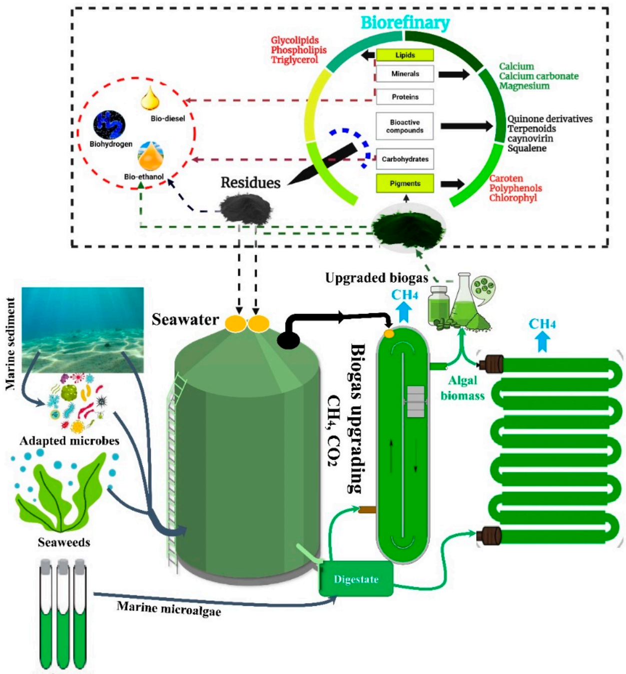

Figure 6. Overview of the suggested approach for integrated marine biorefinery to achieve a zero-waste marine biogas system, where seawater and seaweed could be potentially applied for biogas production integrated with marine microalgae cultivation on the digestate and seawater.

6. Conclusions

Recent studies confirmed the promising potential of seaweed as a biofuel feedstock for anaerobic digestion and biogas production. Though anaerobic digestion efficiency of seaweed needs to be further improved, additional cost-effective technologies such as pretreatment, inhibitor removal, and operational optimization can overcome the technical issues. The present article introduces a new integrated marine-based co-digestion system with sequential processing of biomass as a potential approach to enhance process efficiency. A marine-based system that includes seaweed, seawater, and adapted anaerobic marine microbes integrated with marine microalgae cultivation could improve the quality of the produced biogas, lower CO_2 emissions, and ensure a zero-waste strategy. Although anaerobic co-digestion of seaweed with other biomass feedstocks showed promising results, liquid co-digestion is still not definitive but is worthy of further investigations and optimization.

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