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Assessment of the Seasonal Potential of Macroalgae and Grass in the Sea of Azov for Methanogenesis and Optimization of the Digestate's Carbon/Nitrogen Ratio

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Abstract: Large amounts of macroalgae and grass are dumped on the shores of the Sea of Azov in different seasons. Aquatic plant biomass management could contribute to sustainable development. By mixing them with co-substrates in an anaerobic bioreactor, not only can biogas be extracted, but suitable fertilizers can also be obtained. This study discusses the possibility of using methanogenesis waste from Azov Sea algae and sea grass as a fertilizer for agriculture. The main criterion is the presence of carbon (C) and nitrogen (N) in the waste products of methanogenesis. The influence of climatic and seasonal factors in the Azov region on the quality and quantity of storm emissions, on the productivity of methanogenesis, and changes in the ratio of carbon (C) and nitrogen (N) during methanogenesis and in the fermented substrate has been established. The influence of the ratio of the components of the mixture in various proportions, before methanogenesis, on the productivity of methanogenesis and the change in the ratio of carbon (C) and nitrogen (N) during the process of methanogenesis, and in the fermented substrate were studied. The biomass of the Sea of Azov, cattle manure and wastewater waste in various proportions, were used as components of the mixture. Recommendations are given for the selection of mixture components for methanogenesis, with predicted indicators of the ratio of carbon (C) and nitrogen (N) in the fermented substrate.

Keywords: macroalgae; grass; cattle manure; methane; biogas; energy; soil fertility

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1. Introduction

The process of obtaining biogas from the algae and grass of the Azov Sea is accompanied by the formation of waste. An analysis of recent scientific studies of the metagenesis process indicates that these wastes contain a significant amount of chemicals and elements and can be used as soil fertilizers [1–4]. Osman et al.'s review showed that co-fermentation of macroalgae and seagrass can improve biofuel production, reduce production costs, and enable the optimal use of marine biomass resources [5]. The ability of the soil to create the necessary conditions for the development of agricultural crops and the formation of high yields is inextricably linked with the reserves of organic substances it contains. It is known that humus determines favorable nutritional, water–air, thermal and biological regimes, soil structure, and the accumulation of physiologically active substances. Soils rich in humus are distinguished by the fact that the agricultural crops grown on them are resistant to pathogens and adverse environmental factors and produce products of the highest quality. On such soils, the threat of contamination by toxic substances is reduced, and yields are stable. To maintain the fertile characteristics of the soil, it is necessary to regularly enrich it with fertilizers, thereby providing the crops with the necessary nutritional complex for active growth and strong immunity, and also increasing the quantitative and qualitative characteristics of the crop [6]. Fertilizers made from algae have a number of advantages compared to the most common manure and compost, since they do not contain weed seeds, fungal spores, helminth eggs, and they help improve the structural and mechanical composition of the soil [4].

Numerous experimental and theoretical studies show that the products (waste) of algae methanogenesis are optimal in terms of the main indicator of humus quality (C/N) and are a promising source of organic nitrogen fertilizers [3,4]. A study of the methanogenesis process shows that the best results in terms of humus quality (C/N) are obtained by the fermentation of seaweed, and significantly depend on the conditions (environment) of their cultivation (growing). In this regard, air temperature, sea water, wind speed, and the hydrological regime create unique climatic conditions in the Azov Sea that promote the growth and development of seaweed, where, in addition to phytoplankton, macrophytes and sea grasses are present [7–9]. Due to them, storm emissions are formed, which accumulate both on the shore and in the coastal part of the sea and represent the most suitable source of marine organic matter for methanogenesis.

The process of formation of a nutrient medium for algae and grass depends on the factors described above. Assessing and studying the influence of these factors on the resource base for the process of methanogenesis and methanogenesis products will allow us to formulate the optimal composition of the basic raw material mixture for fermentation.

The C/N value of the fermented substrate depends on the C/N value of the substrate supplied to the bioreactor. Studies conducted on this topic have shown that the optimal C/N ratio for the methanogenesis process is 20–30 [10–12]. However, the C/N value of many macroalgae is lower, usually reaching 10–15 [13,14]. Therefore, macroalgae should be mixed with co-substrates with a higher C/N ratio, such as cow manure, paper waste, corn straw, and wheat straw [14–17]. Sewage sludge is often used as an inoculant in anaerobic digestion [13]. Therefore, by mixing Azov Sea macroalgae in different ratios with cattle manure, litter, and sewage sludge, it would be possible not only to obtain high-quality biogas, but also to achieve optimal C/N values of the fermented substrate. Also, the C/N values of the fermented substrate depend on the construction of the bioreactor. One- or two-stage bioreactors are usually used for anaerobic digestion [18–20]. Studies have shown that high biogas yields can be achieved in a three-stage bioreactor. By using this type of bioreactor, up to 15% more biogas is extracted, and due to its structural features, the energy costs of bioreactor operation are reduced [21,22]. A three-stage bioreactor could be a promising tool for fertilizer production from the excess plant biomass of the Sea of Azov.

Sustainable use of natural resources would contribute to reducing climate change, as the anaerobic digestion of biomass reduces the release of greenhouse gases into the environment. Sustainable management of aquatic plant biomass would improve the protection of the coastal landscape of the Sea of Azov. The use of the surplus biomass growing in water bodies for anaerobic digestion can replace conventional plant cultures. The fertile land managed in this way could be used for the cultivation of food crops.

The purpose of this scientific work is to study the factors influencing the resource potential of storm emissions of algae and grass from the Sea of Azov, used for the process of methanogenesis with the subsequent use of fermentation products as fertilizers. For the first time, an assessment was made of the influence of climatic factors in the Sea of Azov on the effectiveness of methanogenesis. The article describes the prospects for using methanogenesis products, digested in a three-stage bioreactor, as organic fertilizers, taking into account the carbon–nitrogen (C/N) ratio.

2. Materials and Methods

2.1. Quantitative and Qualitative Accounting of Species Composition and the Share of Organic Plant Biomass in Storm Emissions

Observations of storm emissions were carried out from May to November 2021, in the area of the Azov Research Station (ANIS) of the Azov State Technical University, located on the northern side of the Bilosarayska Bay of the Azov Sea (coordinates: 46°56.549′ N, 37°13.171′ E, WGS: 46.94252, 37.21953). The sampling area is presented in Figure 1.

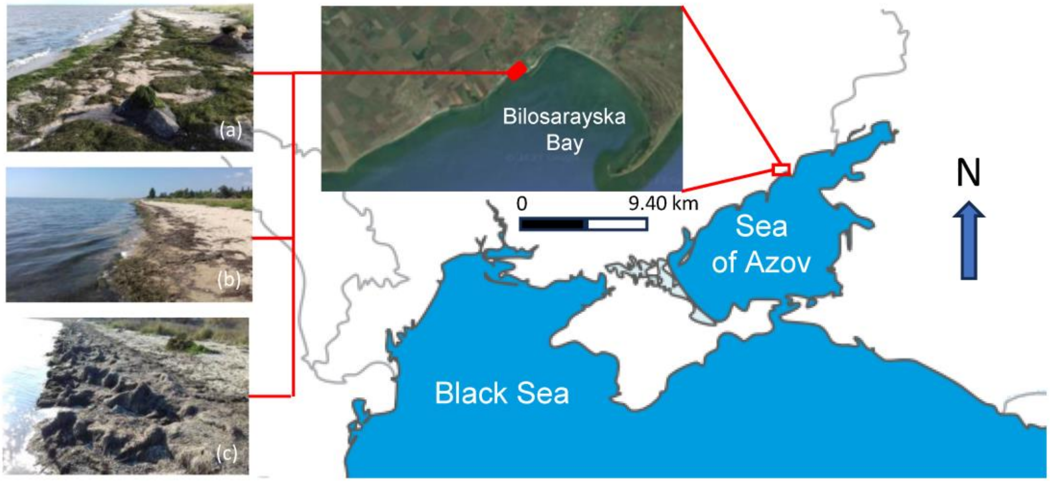


Figure 1. A map of the Sea of Azov region showing the sampling location and photos in different seasons: (**a**) spring; (**b**) summer; (**c**) autumn.

The outbursts were coastal ridges of varying length, mass, and time of formation, while floating clusters of plants could remain near the shore.

Primary processing of the collected material was carried out on the shore immediately after sampling. The samples were washed to remove mineral inclusions (sand, pebbles, shells, etc.) by collecting floating plants from the surface. The qualitative composition of the emissions was determined using the method of photographing each sample, where their composition was clearly visible. After draining (before visible traces of water were removed), the total solid (*TS*) and volatile solid (*VS*) values of the plants were determined.

*TS*s and *VS*s were determined using standard methods [23]. Wet mass (WM) samples were taken for *TS* determination. The mass of each sample was 25 ± 2 mg. The samples were placed in dishes. The plants were weighed to determine the wet weight, and after drying, the dry weight. Drying was carried out in the drying chamber at a temperature of 60–70 °C, until a constant weight was reached. The weighing of the samples was carried out on scales (weighing limit—200 g) with a readout resolution of 0.01 g. *TS* values was calculated according to Equation (1):

$$TS = \frac{W_{total} - W_{dish}}{W_{sample} - W_{dish}} \tag{1}$$

where TS is the total solid value of the plants (g TS/g); W_{total} is the weight of the dried residue and the dish (mg); W_{dish} is the weight of the dish (mg); W_{sample} is the weight of the wet sample and the dish (mg).

The mineralization of the samples was carried out in a muffle furnace at a temperature of 450 °C. The weighing of mineral residues was carried out on scales (weighing limit—220 g) with a readout resolution of 0.001 g. The difference between the dry and mineralized masses was considered to be organic matter. *VS*s were calculated according to Equation (2):

$$VS = \frac{W_{total} - W_{volatile}}{W_{total} - W_{dish}} \tag{2}$$

where VS is the volatile solid value of the plants (g VS/g TS); W_{total} is the weight of the dried residue and the dish (mg); W_{dish} is the weight of the dish (mg); $W_{volatile}$ is the weight of the residue and the dish after ignition (mg).

The values of total solids (*TS*s) and volatile solids (*VS*s) of the plants are presented in Table 1.

Table 1. Average total solid and volatile solid values of plants from storm emissions.

Types of Plants	*TS*, g *TS*/g	*VS*, g *VS*/g *TS*	Samples
Green algae, types: *Enteromorpha intestinalis*, *Enteromorpha clathrata*	0.10 ± 0.02	0.64 ± 0.01	8
Green algae (filamentous), type: *Cladophora albida*	0.17 ± 0.01	0.59 ± 0.01	3
Red algae, types: *Ceramium diaphanum*, *Ceramium rubrum*	0.22 ± 0.03	0.72 ± 0.02	4
Brown algae, genus: *Striaria* sp.	0.18 ± 0.02	0.72 ± 0.02	5
Sea grass, types: *Zostera marina*, *Zostera noltii*	0.18 ± 0.02	0.77 ± 0.02	4

The species composition of the emissions was determined based on the results of photographic recording in laboratory conditions. It is problematic to disassemble the collected mixture into its components in the field due to the large number of small fractions formed as a result of sea surf.

During the observations, green, red, brown algae, and sea grasses were collected and studied, in which the ratio of wet and dry masses, as well as the content of organic matter, was determined (Table 2). The collection is structured according to 3 seasons: spring, summer, and autumn. In each season, 10–15 collections were made for analysis. The data presented in the article is averaged by season. Storm emissions were not collected during the winter season. Sampling during the winter season was not possible due to continuous waterlogging and reduced plant biomass. Therefore, vegetation biomass was not sampled during the winter season.

Table 2. Species composition and organic matter of storm emissions.

Description of Samples by Season	Plant Biomass	Total Dry Organic Matter, %
Spring season	Brown algae: 95%—*Striaria* sp. Green algae: 5%—*Enteromorpha intestinalis*, *E. clathrata*	59.92 ± 0.01
	Brown algae: 50%—*Striaria* sp. Red algae: 50%—*Ceramium diaphanum*, *C. rubrum*	62.04 ± 0.02
	Green algae: 75%—*Enteromorpha intestinalis*, *E. clathrata* Red algae: 25%—*Ceramium diaphanum*, *C. rubrum*	68.03 ± 0.02
	Green algae: 80%—*Enteromorpha intestinalis*, *E. clathrata* Green algae: 5%—*Cladophora albida* Red algae: 15%—*Ceramium diaphanum*, *C. Rubrum*	55.70 ± 0.01
Summer season	Sea grass: 100%—*Zostera marina*, *Z. noltii*	79.59 ± 0.02
	Brown algae: 100%—*Striaria* sp.	71.59 ± 0.02
Autumn season	Brown algae: 66%—*Striaria* sp. Sea grass: 34%—*Zostera marina*, *Z. noltii*	66.77 ± 0.01
	Sea grass: 62%—*Zostera marina*, *Z. noltii* Plant detritus: 38%	61.14 ± 0.01
	Sea grass: 88%—*Zostera marina*, *Z. noltii* Green algae: 12%—*Enteromorpha intestinalis*, *E. clathrata*	59.93 ± 0.01

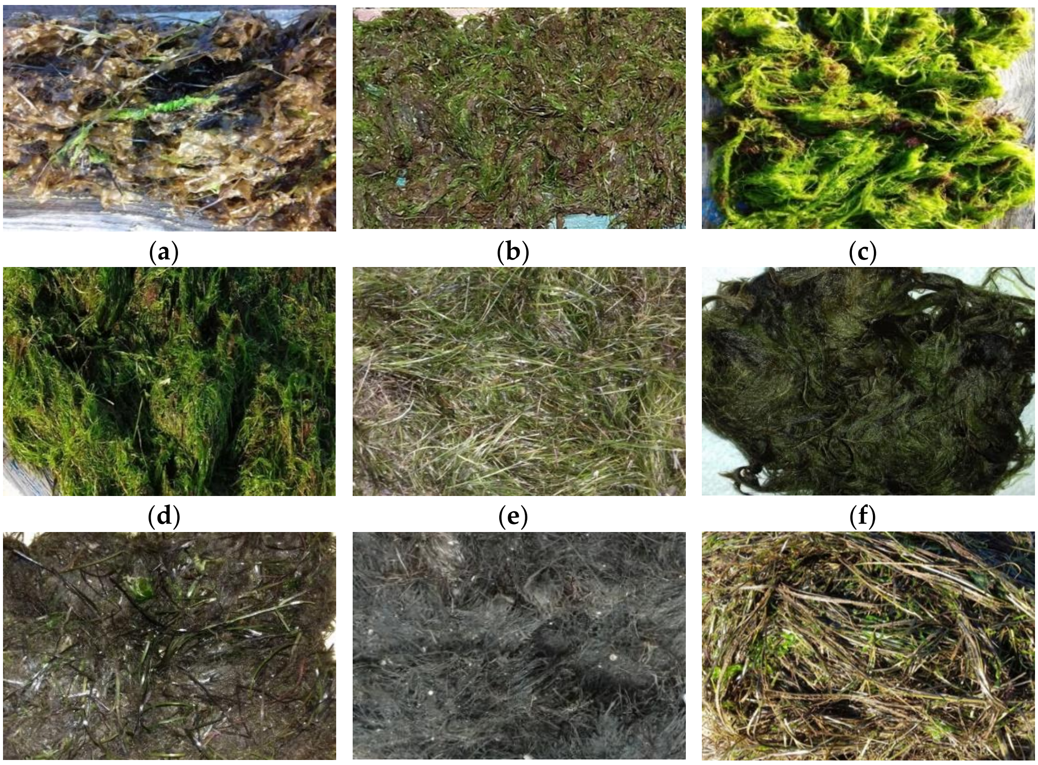
The percentage composition of the biomass of storm emissions was determined using the grid method [7]. This method is used to determine the volumetric composition and structure of natural rocks. The essence of the method is to overlay a grid on a photographic image. Separately, we counted the sum of grid cells that covered green, brown, red and others. These sums were divided by the total number of cells covering the sample, and the image fraction or percentage was determined.

The average composition of plant biomass from storm emissions of the studied samples in the spring–autumn season of 2021 is presented in Table 2.

Table 2 presents the characteristic compositions of samples by season. The composition is different in different samples. The composition is influenced by climatic conditions and seasonality.

2.2. Characteristics of Storm Emissions Samples

Storm emissions were collected over three seasons. Spring samples were collected from March to May 2021. The average air temperature in this season was 18.5 °C. Examples of spring samples are shown in Figure 2a–d. During this season, a southerly wind of varying speeds prevailed. Brown algae, along with red and green varieties, can be found along the shore. Floating algae was collected using a net. A total of 15 samples were collected this season.



(**a**) (**b**) (**c**) (**d**) (**e**) (**f**) (**g**) (**h**) (**i**)

Figure 2. Examples of samples for the three seasons: (**a**–**d**) spring season; (**e**,**f**) summer season; and (**g**–**i**) autumn season.

During the spring season, on the seabed near the shore, algae mats formed, consisting of living green and red algae. Sample collection in the summer season was carried out from June to August; the average temperature was 23.7 °C; 12 samples were collected; an easterly and southeasterly wind prevailed. During this period, there was a frequent increase in winds reaching gale force levels (more than 14 m/s). An example of such sample collection is presented in Figure 2e—strong rush of water. Both floating and shore sea grasses were collected (Figure 2f). Samples were also collected during the summer season; under an unusual southwest wind of 2 points. Algae mats formed near the shore, the bottom of which is black with the smell of H_2S. Floating plants were also collected. Nine samples were collected during the autumn season. Sample collection took place from

September to November. The average temperature was 11.3 °C. An easterly wind prevailed. An example of autumn samples is presented in Figure 2g–i. Figure 2g—complete calm. On the shore, as before, there are emissions formed by easterly winds. Near the shore there are accumulations of floating brown algae on *Zostera marina*—taken from floating plants. Figure 2h—good separation of shells. At the same time, one's hands become contaminated with a sticky small-sized mass that dries out and is difficult to wash off with water. Figure 2i—sunny day, clear water. Easterly wind, 1–2 points. On the shore, as before, there are emissions formed by easterly winds. There are fresh emissions. Near the shore there are mats of *Zostera marina* and green algae. *Zostera marina* serves as a substrate for algae. The sample was taken from the mats.

2.3. Biomass Methanization in a Three-Stage Bioreactor

A substrate composed of aquatic plant biomass (APB), a mixture of cattle manure with wheat straw (CMWS) and sewage sludge (SS) was used to conduct studies of C and N concentrations in the digestate. Cattle manure with wheat straw was used to increase the C/N ratio. The recommended C/N ratio in the substrate is from 20:1 to 30:1. SS was used as an inoculant, which acted as a source of archaea and methanogenic bacteria. The composition of the individual materials is presented in Table 3.

Table 3. The composition of the raw materials supplied to the TSB.

Biomass	*VS*, kg	*TS*, kg	WM, kg	Density, kg/L	WM, L
APB	4.10 ± 0.11	10.9 ± 0.2	71.3 ± 0.2	1.01 ± 0.04	70.6 ± 0.1
CMWS	5.37 ± 0.14	7.8 ± 0.1	50.7 ± 0.2	0.91 ± 0.08	55.7 ± 0.1
SS	1.90 ± 0.10	3.7 ± 0.1	55.0 ± 0.2	1.02 ± 0.06	53.9 ± 0.1
Water	–	–	–	–	119.8 ± 0.1
Total	11.37 ± 0.25	22.4 ± 0.2	296.8 ± 0.3	–	300.0 ± 0.1

The raw materials were crushed and mixed in the preparation chamber, which was installed in the three-stage bioreactor (TSB). Physicochemical parameters were determined when the substrate was mixed. The physicochemical parameters that were determined in the substrate are presented in Table 4.

Table 4. Physicochemical parameters of the substrate.

	TS, g/L	*VS*, g/L	NS, g/L	C/N Ratio	pH
Substrate	80.91 ± 0.32	40.42 ± 0.33	40.51 ± 0.31	26	7.77

TSB was used for digestate studies during the methanogenesis process. The scheme of the TSB bioreactor is presented in Figure 3. The bioreactor consisted of three chambers. In the first 60 L chamber (PC-I), the substrate was homogenized under aerobic conditions. After the first chamber, the substrate was fed to the second chamber with a capacity of 60 L (AAC-II), where it was mixed and heated to a temperature of 20 °C under anaerobic conditions. The methanogenesis process took place under anaerobic conditions in the third chamber with a capacity of 300 L (AC-III). A substrate temperature of 37 °C was maintained in the chamber. In the second and third chambers, the substrate was stirred at a speed of 5 rpm for 5 min per hour.

The substrate in the continuous TSB was fermented for 15 days until the methanogenesis process was established. Changes (Δ) of C and N in the digestate were determined as a result of these studies. The concentrations of C and N were determined using an EA 3000 elemental composition analyzer (Eurovector, Pavia, Italy). The research results showed that, in the three-stage bioreactor, ΔC reached 10.0 ± 0.03 g/kg *TS* d and ΔN—0.3 ± 0.001 g/kg *TS* d. These results were obtained when the initial Cbefore of the substrate supplied to the TSB was 650.0 ± 19.5 g/kg *TS*, and the Nbefore was

25.0 ± 0.1 g/kg *TS* [22]. Using these data, the prospects for the use of methanogenesis products as organic fertilizers were evaluated, taking into account the carbon-to-nitrogen (C/N) ratio of the digested substrate in the TSB.

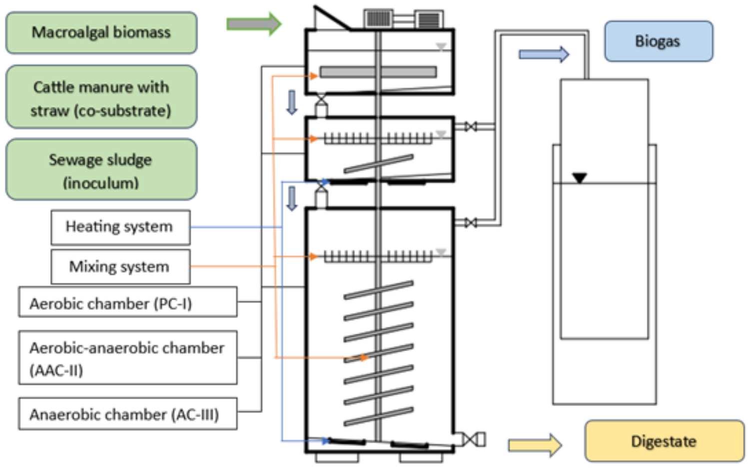


Figure 3. A three-stage bioreactor stand.

2.4. Statistical Analysis

Statistical methods were used for statistical evaluation of the measured data. Substrate composition was statistically evaluated using the following methods. Each measurement was repeated three times. After the measurements, the arithmetic mean and variance of the individual measurements were calculated, which corresponded to the probability distribution of the individual measurements. The experimental standard deviation of the arithmetic mean and the coefficient of variation were also calculated. The coefficient of variation was calculated to check the dispersion of the results. The test of honest significant difference was used to determine the significance of the difference between the analyzed variables. Statistical significance was determined when $p < 0.05$. Microsoft Office Exel 2019 (Microsoft, Washington, DC, USA) was used to perform the statistical calculations.

3. Results

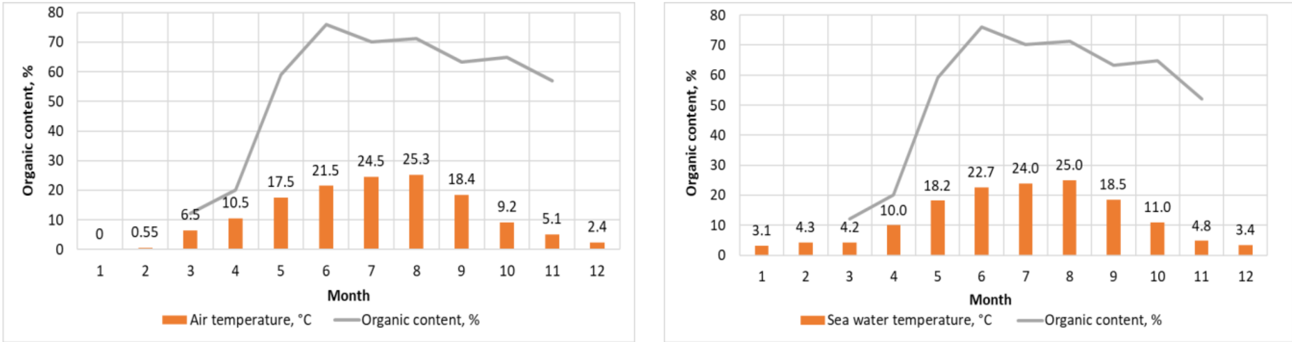
Climatic factors influencing the resource potential of storm emissions of plant biomass in the Sea of Azov include light conditions, water and air temperatures, wind speed and direction, and season. These factors influence the ratio of carbon to nitrogen (C/N), both during the process of methanogenesis and in its residual products.

3.1. Influence of Lighting and Temperature Conditions

The Sea of Azov is the shallowest sea in the world. Its area reaches 37,805 km^2, and the maximum depth is only 15 m. Therefore, the number of sunny days greatly affects the growth of algae and grass in the sea.

For algae, as autotrophic organisms, light, as a source of energy and as a regulator of development, determines the intensity of photosynthesis, the increase in algae biomass, and also determines their vertical distribution in the water of the bay.

Studies have shown that the highest quantity of biomass is achieved during the summer months, when the number of sunny days is greatest (Figure 4a,b). Some researchers place the temperature factor in second place after light. No fewer experts consider temperature to be the first factor determining the periodicity in the development of algae. [24,25]. The relationship between air temperature and water temperature in marine ecosystems is an aspect that has a direct impact on the qualitative composition of seaweeds [26–29].



(**a**) (**b**)

Figure 4. Effect of temperature on the amount of organic matter in algae and grass: (**a**) Effect of air temperature on the amount of organic matter in algae and grass; (**b**) influence of sea water temperature on the amount of organic matter in algae and grass. All values are significant at $p < 0.05$.

As a result of analyzing the data from meteorological indicators in the Azov region for the shallow waters of Taganrog Bay, the difference between water and air temperatures fluctuates very slightly (Figure 4a,b). Thus, in the study period, it did not exceed 3.4 °C in winter, 1.8 °C in spring, 0.9 °C in summer, and 2.1 °C in autumn. The maximum values of air and sea water temperatures (Figure 4a,b) were noted in August (25.3 °C and 25.0 °C, respectively).

It was found that the determining factor in the species composition of storm emissions is also the seasonality of plant growth in the bay. Thus, the composition of storm emissions in the spring period of the year is represented mainly by brown algae (*Striaria* sp., about 95%), and to a lesser extent by sea grass and green and red macrophytes (5.0%). The amount of organic matter in the samples gradually increases from 12.1 to 59.1% as temperatures increase to 17–19 °C (Figure 4a,b).

In general, air and water temperatures have a beneficial effect on the growth of organic matter in algae and grass. Maximum growth rates are observed from June to August. There is no sharp decline observed in the autumn months.

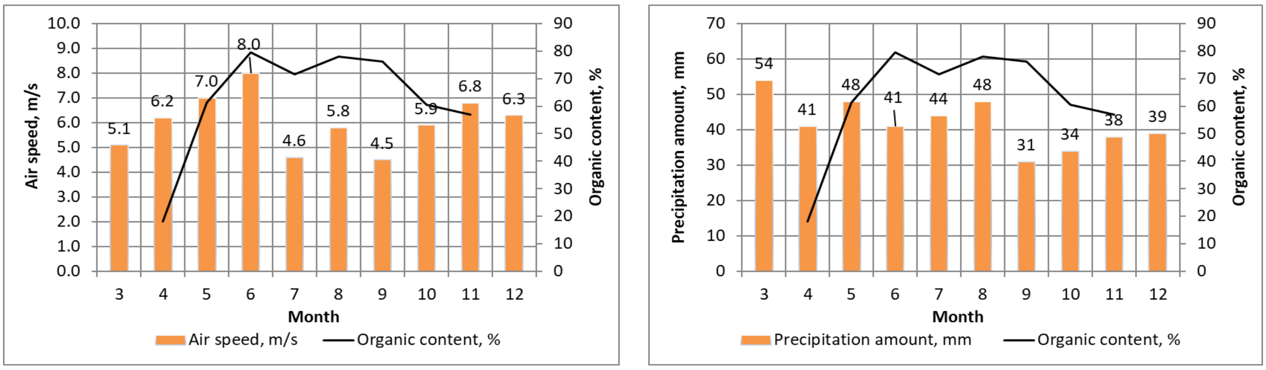
In general, air and water temperatures affect the amount of organic matter in emissions. Maximum growth rates are observed from June to August. However, no sharp decline in this indicator was observed in the autumn months.

3.2. Influence of Wind Speed and Direction

The work investigated the influence of the hydrodynamic regime of water on the chemical composition of algae. The hydrodynamic regime affects the depth of movement, the rate of respiration, and the intensity of phosphorus absorption [30–34]. It has been established that during the warm period of the year, southerly and southwesterly winds dominate in the territory under consideration; in cold periods, easterly and northeasterly winds predominate. Winds with a speed of 5.0–6.5 m/s—i.e., moderate winds—are the most frequent (50%). Strong winds with a speed of 7.0–8.0 m/s or more were observed in the winter months (25%). The effect of air speed and precipitation on the amount of organic matter in algae and sea grass is shown in Figure 5a,b.

The positive influence of hydrodynamic factors on the productivity of algae and grass is manifested in deep-sea reservoirs and consists of a better supply of cells with nutrients, i.e., it is closely related to the dynamics of their water masses: convection currents, wind activity, etc. In shallow parts of the bay, water movement increases turbidity, which negatively affects the development of bottom plants.

Analysis of the composition of organic dry matter showed that the carbon content in the samples fluctuates slightly (27.8–30.7%) throughout all seasons. And the percentage of nitrogen in the organic component acquires maximum values (2.3–2.4%) and a stable composition during the period of the highest temperatures (July–September) (Figure 6). Thus, the change in the total N content in the organic composition of algae and grass is decisive for further studies of the C/N dynamics in the fermented substrate used as fertilizers.



(**a**) (**b**)

Figure 5. Effect of climate on the amount of organic matter in algae and grass: (**a**) Effect of air speed and precipitation on the amount of organic matter in the biomass; (**b**) multifactorial influence of climate on the amount of organic matter in algae and grass. All values are significant at $p < 0.05$.

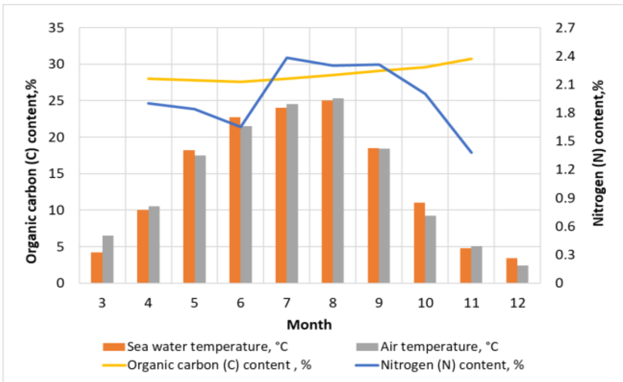


Figure 6. Multifactorial influence of climate on the amount of organic matter in biomass and carbon and nitrogen content in the organic composition of plant biomass by season. All values are significant at $p < 0.05$.

A study of the dynamics of the distribution of organic nitrogen in the process of producing fertilizers by methanogenesis was carried out using the example of macroalgae and sea grass and is presented in Figure 7a,b. The samples corresponded to four variants of raw material samples before methanogenesis. The samples differ in the percentage of macroalgae (Figure 7a) and *Zostera* sp. sea grass (Figure 7b). The percentage composition of aquatic plants in the substrate was: 40, 30, 20 and 10%.

The substrate for methanogenesis consisted of storm emissions, manure, and wastewater. When mixing algae or sea grass with manure and wastewater, the nitrogen content in the mixture increases. During methanogenesis, part of the nitrogen from the substrate is removed with biogas in the form of ammonia compounds, which leads to a decrease in the nitrogen content of the fermented substrate. The work also carried out studies of the dependence of the carbon coefficient (C/N) in the residue with different percentages of storm emissions and manure in the feedstock. The dynamics of changes in the C/N coefficient depending on the season and mixture composition were analyzed during the process of methanogenesis. The dynamics of changes in the C/N coefficient depending on the season and mixture composition were analyzed during the process of methanogenesis and are shown in Figures 8 and 9.

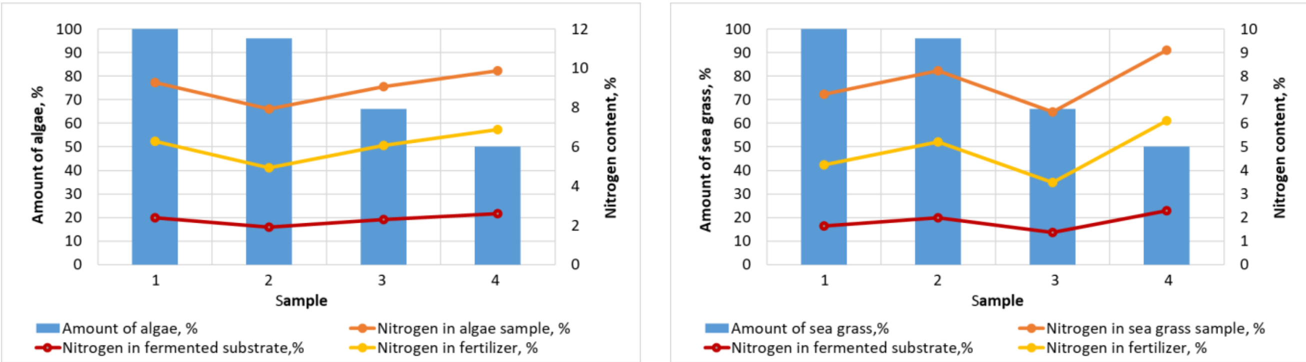


Figure 7. Nitrogen content in the methanogenesis cycle: (**a**) of algae; (**b**) of sea grass. All values are significant at $p < 0.05$.

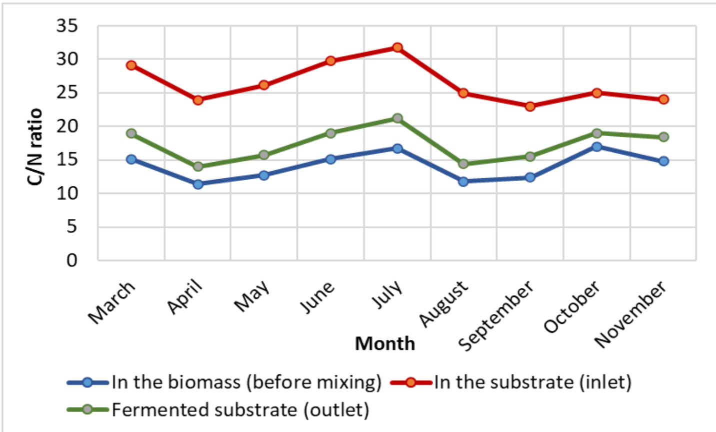


Figure 8. Changes in the carbon coefficient of the substrate and the fermented substrate depending on seasonality and mixture composition before mixing. All values are significant at $p < 0.05$.

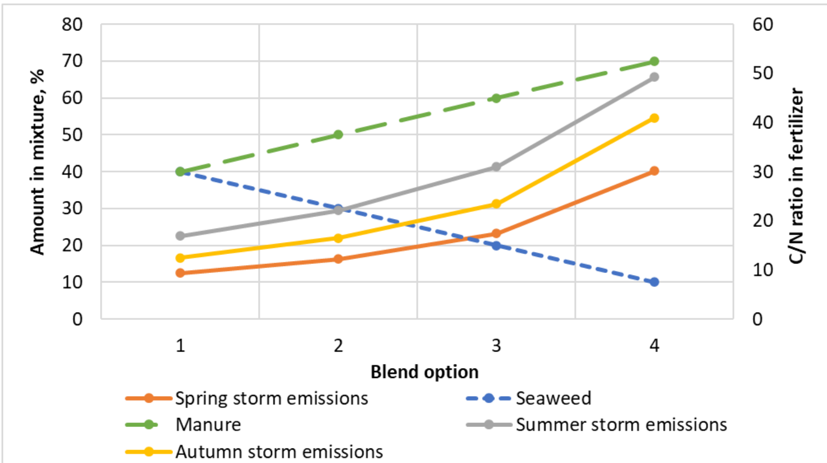


Figure 9. Dependence of C/N ratio (fermented substrate) on the composition of the mixture and seasonality. All values are significant at $p < 0.05$.

Of greatest interest is the possibility of selecting a resource mixture for methanogenesis, providing a given carbon coefficient in the fermented substrate. For this purpose, studies were carried out on the influence of the percentage ratio of the components of the initial substrate mixture on the resulting carbon coefficient of the fermented substrate. The research results showed that the C/N ratio is not the same in different months. The

C/N ratio of aquatic plants before mixing with co-substrates was 10–16. The highest C/N ratios were found in July and October. The C/N ratio was increased by mixing aquatic plants with co-substrates. Cattle manure with straw and activated sewage sludge increased the C/N ratio to 23–31. The obtained results showed that the C/N in the fermented substrate depends on the C/N of the substrate supplied to the TSB and the seasonality.

Research has shown that summer storm emissions give the maximum values of carbon-to-nitrogen ratios in the fertilizer. The influence of algae or grass in the mixture before methanogenesis on the C/N ratio in the fermented substrate has been established, which allows one to select the optimal composition of the mixture for methanogenesis. The obtained results showed that by changing the mixing proportions of macroalgae, cattle manure with litter, and sewage sludge, it is possible to predict the C/N ratio in the digested substrate. The obtained results are presented in Table 5.

Table 5. The characteristic of changes in the C/N coefficient depending on the season and mixture composition.

Blend Option, No.	Amount in Mixture, % (Input)			C/N, in Digestate (Output)		
	Algae/Grass	Manure with Straw	Inoculant	Spring Storm Emissions	Summer Storm Emissions	Autumn Storm Emissions
1	40	40	20	12.5 ± 0.5	16.9 ± 0.5	12.5 ± 0.5
2	30	50	20	16.3 ± 0.5	22.1 ± 0.5	16.5 ± 0.5
3	20	60	20	23.2 ± 0.6	31.0 ± 0.5	23.5 ± 0.5
4	10	70	20	40.2 ± 0.6	49.3 ± 0.6	41.0 ± 0.6

The carbon to nitrogen (C/N) ratio for plant fertilization can vary depending on several factors, including plant type, soil conditions, and stage of growth. A C/N ratio between 10:1 and 30:1 is usually recommended for plant fertilization. Such a C/N ratio ensures a good balance between carbon, which helps maintain soil structure and microbial activity, and nitrogen, which is necessary for plant growth and chlorophyll production. Increasing the amount of algae and grass supplied to the bioreactor decreases the digestate C/N ratio. This is due to the lower C/N ratio of algae or grass than the cattle manure with wheat straw. The results showed (Table 5) favorable fertilization results of the digestate when the algae and grass were collected during all three seasons. The highest C/N values in the digestate were determined during the summer season. Nutrient levels and the stability of digestate can be time-dependent, so it is recommended to use digestate for fertilization in as short a time as possible.

4. Discussion

The study of the resource base of algae and grass in the Sea of Azov was carried out on the basis of qualitative and quantitative analysis of storm emissions in the area of the northern side of the Bilosarayska Bay of the Sea of Azov. Accumulations of storm emissions were formed on the shore in the form of ramparts and along the coastline in the water.

Research has shown that the largest amount of biomass in the Sea of Azov is generated in the summer season. This is determined by the number of sunny days and the temperature favorable for biomass growth. Experimental works have shown that the increase in algae biomass is proportional to the amount of absorbed light up to 25–55 cal/cm^2/day [8,35,36]. For most algae in the waters of Taganrog Bay, the optimum level of light lies in the range of 20–50% of the incident visible radiation, but for some it can differ significantly from these indicators [37].

Under natural conditions, the intensity of illumination, as it is known, varies not only throughout the year, but also during the day. The light regime of the bay water area depends on: lighting conditions above the water surface; the degree of absorption and scattering of rays when passing through water; the degree of reflection of light by the water surface. When the sun is high, a smooth water surface reflects on average 6% of the incident light, with strong waves—about 10%, when the sun is low, the reflection increases so significantly that most of the light no longer penetrates into the water [38,39]. The optimal illumination values for individual algae species vary over a fairly wide range. For example, for *Chlorella* sp. they are in the range of 3000–4000 lux, for other green algae—5000–7000 lux. Light conditions in the bay are determined by the transparency of the water. Transparency is understood here as the ratio of the light flux emerging from the water to that incident on its surface. The transparency of water in a reservoir determines both the vertical distribution of algae and the production characteristics of communities [28,29,40–42].

The species and qualitative composition of storm emissions was studied using the photo recording method, taking into account seasonal changes in indicators, based on the analysis of nine samples and revealed green algae: *Enteromorpha intestinalis* and *Enteromorpha clathrat*; green algae (*filamentous*): *Cladophora albida*; red algae: *Ceramium diaphanum* and *Ceramium rubrum*; brown algae: *Striaria* sp.; sea grass: *Zostera marina* and *Zostera noltii*.

The storm discharges of the spring period (samples Nos. 1, 2, 3, and 4 May) are characterized by the predominance of brown algae (60% at the beginning of the season) with a subsequent increase in the proportion of green algae (80%) with the co-growth of red algae (from 5 to 50%). Samples of coastal accumulations of the summer period (samples Nos. 5 and 6—June–July) in July consisted mainly of sea grass (100%), and, in mid-summer, of brown algae (100%), which is explained by the seasonal change in the growth of types of macrophytes, with a spring increase in water temperature and a change in the use of substrate for attachment and zoospores. The main substrate for macrophytes in the Sea of Azov is the sea grass *Zostera* sp., the growing season of which is difficult in winter, and the impact of storms is at its maximum. According to the location from the surface to the bottom, macrophytes are located according to phototaxis: green, brown, then red. This means that, in winter, the degree of storm impact occurs in the same sequence. Therefore, the biomass of green algae at the beginning of the growing season increases (almost from scratch) more slowly than brown and red algae that have overwintered as formed plants on the root areas of sea grass.

By the beginning of the autumn season (samples Nos. 7, 8, and 9—September–November), the proportion of brown algae begins to fall in favor of the active growth of sea grass due to stormy winds cutting off, first the upper and then the lower sections of the growing sea grass. The young upper areas of *Zostera* sp. during this period were not yet covered with macrophytes and therefore consisted of sea grass in the outliers. Subsequently, the brown algae growing below the melted grass began to disappear, and they began to dominate the emissions. Studies of the chemical composition have shown that in storm emissions the dry biomass of the substance is on average 16.6%, and the share of organic matter in it is 68.7%, which allows them to be considered as a raw material for the process of methanogenesis with the production of biogas; therefore, to increase the caloric content of biofuels, it is advisable to use other types of bio-raw materials containing a large amount of organic compounds.

Detailed analysis of the effect of seasonal changes on the organic component of storm emissions showed the need to take into account climatic factors affecting the species and the qualitative composition of marine biomass, and, consequently, the composition of the fermented substrate in order to develop a tailored approach when selecting fertilizers for soils. This is confirmed by the works of other authors [10,33,34,43,44]. This approach consists of the ability to influence the nitrogen and carbon content in the soil through the introduction of a fermented substrate into it. The selection of certain components of the substrate before fermentation allows one to obtain an already-fermented substrate with an optimal ratio of nitrogen and carbon, and to control this ratio. The optimal ratio of nitrogen to carbon in soil depends on various factors, including soil type, climate conditions, and the types of plants grown in that soil. Typically, the optimal ratio of nitrogen to carbon in soil is approximately 10:1 to 20:1. This ratio provides sufficient nitrogen for plants and microorganisms, but does not result in excess nitrogen, which can pollute the environment. The optimal ratio may vary depending on specific conditions [6].

The influence of climatic parameters on the qualitative and quantitative composition of storm emissions was analyzed to ensure the selection of substrate components that provide a given ratio of carbon and nitrogen (C/N) in the fermented substrate and in further fertilizer. A number of authors have shown that this indicator depends on the composition of the substrate, the type of macroalgae [14,45], the conditions of anaerobic digestion, and the season in which raw materials for methanogenesis are collected. Akila et al. [14] found that for the macroalgae *Ulva* sp., when mixed with cow manure in a ratio of 3:1, the concentration of chemical parameters that determine soil fertility increased up to three times. Zahan et al. [45] found that incorporation of two, three, or four different substrates increases the biodegradability of volatiles due to a synergistic effect. This is also determined by the optimization of the C/N ratio in the substrate. Due to the greater biodegradability of *VS*s, the physicochemical parameters of the digestate, which determine the quality of the fertilizer, improve.

Long-term studies show that the greatest influence on the state of biomass in storm emissions is exerted by light conditions, water and air temperature, wind speed and direction, and seasonality [34].

Since an important component of the characteristics of fertilizer is the ratio of carbon to nitrogen (C/N), changes in indicators in the original substrate and fermented substrate were studied [46–48].

It is known that environmental temperature affects the functional activity of algal cells, accelerating or inhibiting metabolic processes [24].

The temperature of sea water is largely dependent on air temperature as it is exposed to the atmosphere. In the region under consideration, during periods of intensive growth of plant biomass and maximum formation of storm emissions (May–November), average monthly temperature fluctuations were within 12 °C with an average wind speed of 6 m/s.

In autumn, conditions change: the length of daylight hours decreases and the water temperature decreases, which can affect the metabolism of algae and the level of carbon and nitrogen in their cells. Perhaps, at this time of year, there is a decrease in the carbon and nitrogen content in algae. The C/N content of algae may also depend on the availability of nutrients in the aquatic environment, which may vary seasonally. Fluctuations in the content of carbon and nitrogen are possible depending on their availability for algae [29]. Understanding these seasonal changes in algae or grass C/N ratios is important for better understanding the ecological processes of marine ecosystems and their response to seasonal changes in climate and nutrients. These studies can help predict and explain changes in biomass and biodiversity in marine ecosystems.

An increase in air temperature leads to the heating of the surface layers of sea water, increasing the intensity of heat exchange and heat transfer from air to water and back. This leads to the intensive growth of algae in the bay and an increase in storm emissions.

With increasing water and air temperatures, there was a tendency for the photosynthetic activity of algae to increase, and higher temperatures corresponded to larger upper limits of photosynthesis. In shallow areas, a direct dependence of the photosynthetic activity of algae on the temperature indicator was revealed [28]. According to our observations, it is the temperature factor that determines the seasonal change in the growth of plant biomass in Taganrog Bay.

Air speed is relatively stable throughout the year; however, there is a correlation between even a slight increase in air speed and an increase in the growth of the organic part of plant biomass. The speed of air movement has a greater influence on the quantitative parameters of the samples. Thus, with significant mixing of water, the amount of brown algae in storm emissions increases, since in calm water these algae, having a relatively heavy substrate, stay near the bottom.

Controlling the percentage of the mixture allows one to select the composition of the fermented substrate with the carbon coefficient required by the agricultural consumer, and solves the agrotechnical problems. In order to implement this approach, various percentages of mixtures were considered and the optimal option for a given carbon coefficient was selected.

5. Conclusions

The work assesses the influence of climatic factors on the resource potential of storm emissions of plant biomass in the Azov Sea. The percentage composition of the biomass of storm emissions was determined using the grid method. Light conditions are considered a WAG factor, as are water and air temperature, wind speed and direction, and season of the year. As a result of methanogenesis using storm emissions from the Sea of Azov, in addition to biogas, a fermented substrate is formed, which is effective when used as a fertilizer. Along with the study of the potential possibility of using storm emissions from the Sea of Azov as a raw material for the production of biogas, the characteristics of the product of methanogenesis—a fermented substrate for use as a fertilizer—were studied. The effectiveness of the fermented substrate as a fertilizer has been proven based on the presence of carbon and nitrogen as its main components, affecting soil fertility. The connection between climatic and seasonal factors and the components of the mixture before methanogenesis on the formation of the optimal ratio of carbon and nitrogen in the fermented substrate are shown, which allow one to effectively influence soil fertility. The sustainable use of the biomass of plants growing in the Sea of Azov could contribute to the goals of sustainable development. During the fertilizer production process, biogas would be obtained, the use of which would reduce the release of greenhouse pollutants into the environment. Sustainable management of biomass would improve the protection of the coastal landscape of the Sea of Azov.

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