Ensiling of Sargassum for Biogas Applications

Effects of Dry Matter Content and Additives on Fermentation Dynamics and Biomass Preservation

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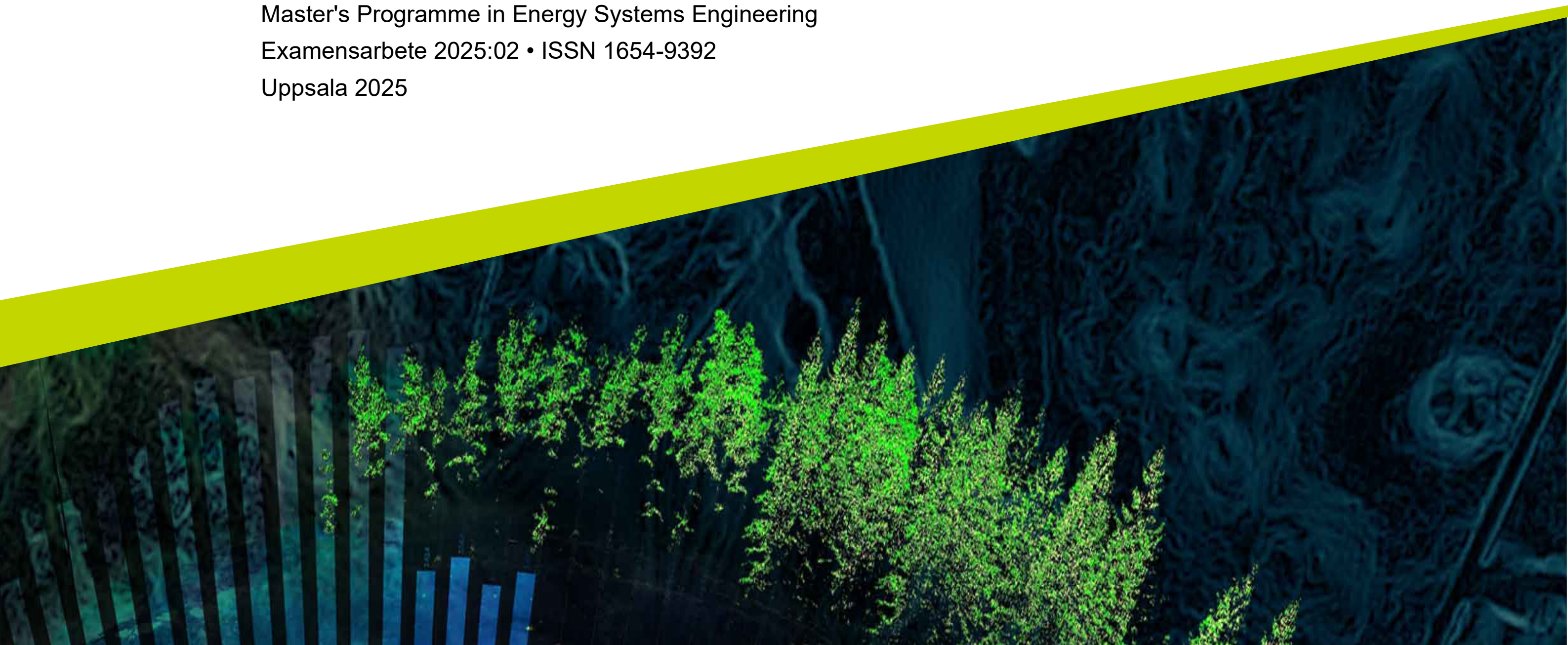

Ensiling of Sargassum for Biogas Applications. Effects of Dry Matter Content and Additives on Fermentation Dynamics and Biomass Preservation

Ensilering av Sargassum för biogastillämpningar: Effekter av torrsubstanshalt och tillsatser på fermenteringsdynamik och bevarandet av biomassa

Terése Nyström

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Abstract

Grenada, a small island nation in the southeastern Caribbean, faces challenges regarding energy dependency and environmental disruption from recurring Sargassum seaweed influxes. While Sargassum accumulation poses ecological and economic issues, it also offers the opportunity as renewable feedstock for biogas production via anaerobic digestion. Due to the seasonal nature of the sargassum, effective long-term preservation is critical to ensure year-round feedstock availability.

This study evaluated the effects of different preservation methods, ensiling with or without silage agents, sugar supplementation, and varying dry matter contents, on the stability of Sargassum over an 8-week storage period. Key parameters such as pH, total solids (TS), and volatile solids (VS) were measured for six treatment groups. Additionally, a mathematical model was developed to estimate VS degradation over a six month period.

The results showed that Sargassum ensiled at 30% DM with a silage agent (30DM) achieved the most favorable outcomes: a rapid pH drop to 4.4, lowest VS loss of 11%, and stable TS content. In contrast, untreated biomass (CTRL) exhibited the steepest degradation, with VS declining from 60% to 37% and a final pH of 5.8, indicating poor preservation. The predictive model shows an increasing divergence between treatments over time. The model predicted that the 30DM treatment would maintain VS levels around 48% after 26 weeks, while the CTRL group would drop below 33%.

These findings highlight the potential of ensiling, particularly at 30% DM with additive, as a practical and scalable method for Sargassum preservation. Implementing such strategies can help stabilize biogas feedstock supply and support Grenada's transition to a more sustainable energy system.

Populärvetenskaplig sammanfattning

Tänk dig att din lokala strand varje sommar täcks av ett brunt, illaluktande sjok av ruttnande tång. Det är verkligheten för många invånare i Karibien, särskilt på önationen Grenada där enorma mängder av brunalgen sargassum spolas upp längs kusterna. Det här är inte vilken tång som helst. Den flyter fritt över Atlanten i enorma bälten, och sedan 2011 har förekomsten ökat dramatiskt, med stora ekologiska och ekonomiska konsekvenser.

Ett annat problem Grenada står inför är att de idag nästan helt beroende av importerad olja för sin energiförsörjning. Det är dyrt, osäkert och långt ifrån hållbart. Samtidigt efterfrågas förnybara energikällor som minskar både klimatpåverkan och sårbarhet gällande energiförsörjning. En intressant lösning som undersöks på flera håll i världen är biogas. Som går ut på att ta tillvara på organiskt material och genom en naturlig jäsningsprocess omvandla det till metan som kan användas för el, värme eller fordonsgas.

Men sargassum är en utmanande råvara då den endast driver i land delar av året. Den bryts även ner snabbt när den hanmar på land och måste bevaras på något sätt för att kunna användas året runt. En nyckelfråga blir därför: Hur kan man lagra sargassum på ett sätt som både bevarar dess energiinnehåll och fungerar praktiskt i ett tropiskt småskaligt sammanhang?

Det är just det denna studie har undersökt. Genom försök både i fält på Grenada och i labbmiljö i Sverige testades olika metoder för att lagra sargassum över tid. En särskilt intressant metod är ensilering, samma teknik som används för att konservera exempelvis vall till djurfoder. Där får mikroorganismer i syrefri miljö sänka pH-värdet och skapa en sur miljö som hämmar förruttnelse.

I experimenten testades hur olika behandlingar, till exempel tillsats av mjölksyrabakterier, socker och olika torrsubstanshalter påverkade lagringsstabiliteten. Resultaten visade tydligt att sargassum som lämnades obehandlad snabbt förlorade sitt energiinnehåll. Däremot kunde man, genom att tillsätta både bakterier och en lagom mängd socker, uppnå en kraftig pH-sänkning och bevara mer av det organiska materialet. Allra bäst fungerade en kombination där algen torkats till 30 % torrsubstans innan ensilering. Resultatet visade då att fukthalt, syrahalt och energivärde bevarades bäst under hela lagringsperioden.

För att förstå vad som skulle hända över ännu längre tid, användes också en matematisk modell som kunde förutsäga nedbrytningen av det organiska materialet över sex månader. Modellen visade att skillnaderna mellan de olika behandlingarna ökade över tid och att de bästa metoderna kunde halvera förlusten av energirikt material jämfört med den obehandlade sargassumen.

Sammantaget visar studien att sargassum inte bara är ett problem att bekämpa utan det är en resurs som kan tas tillvara på. Med rätt teknik går det att minska nedbrytnningen av det och förhoppningsvis använda den som ett lokalt producerat förnybart bränsle. Det kan bli en viktig pusselbit i Grenadas energiomställning och kanske ett exempel för andra kustsamhällen som står inför liknande utmaningar.

Exekutiv sammanfattning

Detta examensarbete har undersökt hur den invasiva brunalgen Sargassum, som i ökande mängder driver iland på Grenadas kuster, kan bevaras som substrat för biogasproduktion. Bakgrunden är landets stora beroende av importerade fossila bränslen och behovet av lokala, förnybara energikällor.

Fyra olika lagringsmetoder testades i laboratorieförsök, med fokus på ensileringstekniker och variationer i torrsubstanshalt samt tillsats av mikrobiella ensileringsmedel och socker. Resultaten visade att obehandlad biomassa snabbt förlorade sitt energiinnehåll, medan kombinationen av ensileringsmedel och en torrsubstanshalt på 30 % gav bäst bevarandeeffekt, stabil pH-utveckling och minskad nedbrytning av organiskt material. En kompletterande matematisk modell med avstamp i mätvärderna visade att minskningen av organsik materia även bör mattas av vid längre lagringstider.

Med avstamp i den här studien rekomenderas att undersöka längre lagringstider av sargassum med en koncentration av vattenlösliga socker. Detta genom ökad torrsubstans som visats i den här studien eller genom tillsättning av sockerrika substart. Det rekomenderas även att genomföra satsvisa biogastester för att undersöka hur ensilering påverkar metanproduktionen.

Abbreviations

DM	Dry Matter
TS	Total Solids
VS	Volatile Solids
HHV	Higher Heating Value
LAB	Lactic Acid Bacteria
AD	Anaerobic Digestion

Contents

1 Introduction

Across the world, the need for sustainable energy solutions has become one of the most defining challenges of our time. As greenhouse gas emissions start to show real consequences in the form of climate change, the global energy system is being reimagined to prioritize renewable resources, circular economy principles, and local production. This transformation is particularly vital for small islands, which are often the first to experience the environmental and economic vulnerabilities linked to climate instability (United Nations Development Programme 2024). These nations, though minor contributors to global emissions, often find it difficult to transition toward sustainable energy solutions.

These challenges are particularly evident in Grenada, a small island nation in the southeastern Caribbean. With limited domestic energy resources, the country almost entirely depends on imported fossil fuels to power its economy (International Renewable Energy Agency (IRENA) 2023). This dependency not only imposes a substantial financial burden, but also leaves the nation vulnerable to supply chain disruptions and geopolitical tensions. In addition to its energy challenges, Grenada is also facing increasing environmental pressure, particularly due to the increasing flow of Sargassum, a floating brown macroalgae that has been washing up on the country's shores with greater frequency and volume in recent years (Gray et al. 2021).

Although Sargassum plays an important ecological role in the environment by offering habitat, food and nursery space for marine life, its mass accumulation along Caribbean coasts since 2011 has created a suite of environmental, economic, and public health challenges (Gray et al. 2021; Pries et al. 2023). The area is forces to take care of decaying mats of algae that negatively affects fishing activities and tourism.

The larger amounts of sargassum have the potential to be utilized as a substrate for biogas production. This aligns with a broader global interest in utilizing biological materials for sustainable energy generation, reducing reliance on fossil fuels, and managing organic waste streams. AlgaeFuel Sweden is actively exploring the feasibility of converting biomass from sargassum into sustainable energy sources. However, a major obstacle in optimizing year around biogas production lies in effectively storing the biomass in a way that preserves its quality over time, given the seasonal availability of sargassum.

1.1 Aim

The overall aim of this study is to investigate practical, scalable, and sustainable methods for long-term storage of Sargassum on Grenada. These storage strategies are intended to ensure a stable and continuous supply of biomass for biogas production throughout the year, thus supporting Grenada's transition to renewable energy. More specifically, the study seeks to improve the understanding of how different storage and treatment methods affect the preservation and biogas potential of Sargassum over time. By evaluating and refining these storage techniques, the project also aims to promote the sustainable and economically viable use of Sargassum as a renewable energy resource.

1.2 Research Questions

1. To what extent do variations in ensiling conditions affect the conservation of volatile solids and the energy yield potential of sargasum for biogas applications?
2. How does the addition of sugar and/or silage agent influence the short-term pH dynamics during the initial hours of the ensiling process of Sargassum?
3. How can the degradation of volatile solids (VS) in Sargassum be estimated over a six-month storage period under different ensiling treatments, and what do these projections reveal about the long-term preservation potential of each method?

1.3 Scope and Limitations

This study focuses on evaluating selected storage and pretreatment methods for Sargassum over a six-month period under controlled conditions. It does not include economic assessments, or large-scale implementation. The results are limited to the preservation of biomass quality for biogas production and do not account for long-term environmental or policy considerations.

2 Background/Theory

2.1 Energy System in Grenada

Grenada is the southernmost island nation in the Lesser Antilles, located in the eastern Caribbean Sea (Encyclopædia Britannica n.d.). Despite its small size of 348.5 square kilometers, Grenada comprises three inhabited islands: the main island Grenada, as well as Carriacou and Petite Martinique (BBC News 2023; Government of Grenada n.d.). This island nation is highly dependent on external resources, particularly when it comes to energy, which makes the resilience and sustainability of the country's energy system a central concern for development and energy security. The vast majority of Grenada's energy is supplied through imported fossil fuels. In 2021, 93% of the total primary energy supply—amounting to 4737 terajoules (TJ)—was derived from petroleum products (International Renewable Energy Agency (IRENA) 2023). Only 7% of the energy supply came from renewable sources, primarily bioenergy at 95% of the renewable share and with small contributions from solar and wind (Flores and Peralta 2020). This is represented in figure 1. The heavy reliance on fossil fuels results in a low energy self-sufficiency, placing Grenada among the most import-dependent energy systems in the Caribbean (ibid.).

In 2022, 94.2% of the population had access to electricity, and 88.3% had access to clean fuels for cooking in 2021 (International Renewable Energy Agency (IRENA) 2023). However, these positive indicators mask underlying systemic vulnerabilities that comes with its low self-sufficiency. The country imported over 27 million kilograms of refined petroleum in 2023, primarily from the United States, Cayman Islands and Trinidad and Tobago, with a total value of approximately USD 98.5 million (Observatory of Economic Complexity (OEC) 2024; World Bank Group 2023). Refined petroleum was also Grenada's largest import by value, underscoring how energy costs shape the national economy and strain public finances (Central Intelligence Agency 2025).

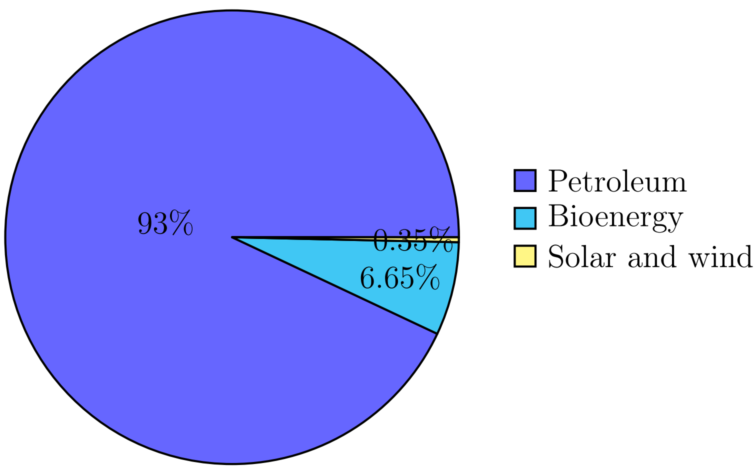

Figure 1: Energy mix on Grenada year 2021 ((International Renewable Energy Agency (IRENA) 2023; Flores and Peralta 2020).

Electricity generation in Grenada reflects this fossil fuel dependence. In 2018, the electricity mix consisted of 98.5% diesel-based generation and only 1.5% renewables (U.S. Department of Energy 2020). Installed electricity capacity stood at 50.9 MW, with a peak demand of 33.2 MW, meaning that while the grid was not overstretched, it had little margin for future expansion. Total electricity generation in 2021 was 229.2 GWh or 0.23 TWh (**irenaGrenada**). Electricity services across all three islands are solely provided by Grenada Electricity Services Ltd. (GRENLEC), a company holding an exclusive license to generate, transmit, distribute, and sell electricity until 2073 (Flores and Peralta 2020). GRENLEC operates several diesel power plants, supplemented by smaller-scale solar photovoltaic installations and a 2 MW wind farm located on Carriacou (ibid.). Transmission and distribution losses are estimated at 7.2%, a relatively common figure for island grids but still a source of inefficiency and lost revenue (U.S. Department of Energy 2020).

High electricity prices further exacerbate the situation. As of 2020, the average electricity rate in Grenada was USD 0.32/kWh for residential and commercial users, and USD 0.28/kWh for industrial consumers (ibid.). These prices are significantly higher than the global average on 0.162, and primarily driven by the cost of imported diesel for generation (GlobalPetrolPrices.com 2025). High energy costs pose a major barrier to economic development and energy equity, especially for low-income households and small businesses (Flores and Peralta 2020).

Beyond the economic implications, Grenada's fossil fuel dependence exposes the country to significant risks related to climate change, disasters, and global market shocks. According to Flores and Peralta (ibid.), this dependence limits national investment capacity and development opportunities, increases environmental degradation, and undermines long-term ecosystem resilience. Furthermore, disruptions in the global oil supply—caused by extreme weather events or geopolitical instability can lead to severe energy shortages and price spikes. In a region frequently impacted by hurricanes and other natural disasters, energy security is intrinsically tied to climate resilience. To address these challenges, the overarching goal for the country is to reduce fossil fuel dependence and enhance the island's capacity to withstand external shocks, while contributing to broader climate mitigation goals.

2.2 Sargassum as a Biomass Resource

Macroalgae is a collective term for a variety of different algae, also known as seaweed. Different families and genus have distinct characteristics and can provide various important ecological, environmental, and economic services (Pries et al. 2023; Devault et al. 2021). A noteworthy family of brown macroalgae is sargassaceae, that has a worldwide distribution in tropical, subtropical, and temperate waters with 481 recognized species. The Sargassum genus within the sargassaceae family is the most diversified with 335 species recognized alone (Stiger-Pouvreau, Bourgougnon, and Deslandes 2014). Most species are benthic, meaning that they are anchored to the ocean floor during some stages of their life (Devault et al. 2021). Two of the most recognized benthic species of sargassum is *Sargassum fusiforme* found in the Yellow Sea and the Bohai Sea of China, as well as *Sargassum Muticum* found in waters around Europe (Zhang et al. 2020; Stiger-Pouvreau, Bourgougnon, and Deslandes 2014). However, there are two species of Sargassum that are pelagic and free-floating throughout their entire lifecycle. These are *Sargassum natans* and *Sargassum fluitans*, which until recently was confined to the Gulf of Mexico and Sargasso Sea (Stiger-Pouvreau, Bourgougnon, and Deslandes 2014). In this report sargassum is reference to the two pelagic species *Sargassum natans* and *Sargassum fluitans*.

Sargassum is visually characterized by its cylindrical structure, gas-filled bladders (pneumatocysts), and leaf-like blades. According to Pries et al. (2023), these characteristics and its flexible body allow the sargassum to flow freely and withstand stress of ocean currents. The two species

Sargassum natans and *Sargassum fluitans* have some visual distinctions that can be seen in figure 2. *Sargassum fluitans* are characterized by their oblong bladders without spines, as well as thorns on the stem. In contrast, *Sargassum natans* has spherical bladders that may have spines depending on the subspecies, as well as a smooth stem (Lambert et al. 2024). However, even if these are two different species, they live and grow together.

Since 2011, there has been a significant increase in pelagic Sargassum biomass across the tropical Atlantic Ocean. This expansion has culminated in the formation of the Great Atlantic Sargassum Belt (GASB), an extensive floating aggregation stretching approximately 8,850 km from the west coast of Africa to the Gulf of Mexico (Pries et al. 2023). Evidence suggests that a considerable proportion of the Sargassum entering the Gulf of Mexico originates from the North Equatorial Recirculation Region, a key pelagic bloom zone in the central Atlantic (Franks and Johnson 2018; Pries et al. 2023). Satellite measurements indicate that Sargassum blooms initiate in the central western Atlantic between February and March. These biomass accumulations are subsequently transported westward by winds and surface currents, trapping Sargassum in the North Brazil Current Retroflection (Franks and Johnson 2018). Some escape by swirling motions in the water, so called eddy motions, or in other ways break free from the North Brazil Current Retroflection (Franks and Johnson 2018; Childs 2011). These later impacts the Lesser Antilles, the long arc of small islands in the Caribbean Sea, resulting in an inflow of sargassum between April to August (Gray et al. 2021; Encyclopædia Britannica n.d.; Franks and Johnson 2018). However, the 2018 and 2019 season lasted almost until the end of the year, leading to an extended inflow period of Sargassum (Gray et al. 2021). The southern Lesser Antilles are particularly vulnerable to early season influxes due to their geographical position within its widespread current pathways (Franks and Johnson 2018). As this phenomenon has only emerged in recent years it presents considerable challenges for the nations impacted by the massive influxes.

Sargassum has been present in the Lesser Antilles every year since 2011 and nothing indicates that it will stop in the near future (Gray et al. 2021). When climatic conditions are favorable, Sargassum reproduces and accumulates biomass rapidly. They reproduce only asexually through fermentation, where an individual sargassum breaks down into two or more parts, each of which continues to live and grow. They double in size every 9-13 days (Pries et al. 2023). Several factors contribute to the increased growth and accumulation of Sargassum, including coastal eutrophication, hurricane activity, nutrient discharges from major river systems such as the Amazon and Congo, and broader impacts of climate change (Milledge and Harvey 2016b; Gray et al. 2021). Climate change is projected to drive a range of oceanic changes that favor Sargassum proliferation. Rising ocean temperatures can extend the duration of the growth season and expand the geographical range where Sargassum can thrive (**kumar2020**). Additionally, coastal eutrophication caused by the enrichment of water bodies with nutrients due to activities like agricultural runoff, sewage discharge, and industrial effluents, significantly enhances the availability of nitrogen and phosphorus. These conditions create an ideal environment for the accelerated growth of Sargassum, resulting in larger and more persistent blooms (Devlin and Brodie 2023).

(a) Sargassum Fluitans

(b) Sargassum Natans

Figure 2: The two pelagic species of Sargassum.

These massive blooms of Sargassum are associated with many issues regarding economics, environment, and human health and welfare (Gray et al. 2021). There are two parts when it comes to issues regarding sargassum, the first is the impact it has on marine life. For example, when seaweed increases excessively, it can outcompete native species and smother benthic habitats. In addition, it can block sunlight from reaching marine photosynthetic organisms and thus disrupt delicate marine ecosystems, like coral reefs(Pries et al. 2023). The second is the impact sargassum has when it is washed ashore and accumulates in dense, malodorous heaps. The main issue is when this heaps start to decompose and releases hydrogen sulfide gas, which produces a pungent "rotten egg" odor and contributes to air pollution (Gray et al. 2021). In 2015, the vice-president of the University of the West Indies described the Sargassum phenomenon as "the greatest single threat to the Caribbean," highlighting the urgency and scale of the problem (Pries et al. 2023).

The economic consequences of sargassum are severe in the Caribbean and on the east coast of central America. This region is heavily dependent on coastal tourism and fisheries (Food and Agriculture Organization of the United Nations 2014). According to Pries et al. (2023), the tourism industry is negatively affected by the massive blooms. For instance, in Quintana Roo, Mexico, tourism reportedly fell by an estimated 30–35% due to Sargassum invasions in the area, with some hotels spending upwards of $54,000 per month on removal efforts alone. Beyond tourism, the fishing industry has also experienced disruptions, leading to reduced catch yields and frequent damage to fishing gear. Furthermore, the presence of sargassum and its associated issues discourage investments in new coastal developments (ibid.).

Most issues related to the environment and human health and welfare are a direct consequence of the decomposition of sargassum (ibid.). when decomposing, sargassum emits both toxic gases, such as hydrogen sulfide and ammonia, as well as greenhouse gases (Silva et al. 2023). Environmentally, this contributes to atmospheric pollution and climate change. Hydrogen sulfide is known to react with oxygen in the atmosphere and create sulfur dioxide and other harmful sulfur oxides that cause acid rain (Pudi et al. 2022). Ammonia is the other gas that is emitted in large quantities from decomposition of sargassum. When released to the air, it can have an effect on the surrounding areas such as plants and crops where it burns the leaves (Environment and Climate Change Canada 2021). It is also harmful for some aquatic species when released to water, even in smaller quantities. An exposure lasting over a few hours can be lethal (ibid.). The negative affect on human health and welfare are also related to the emissions of hydrogen sulfide and ammonia. In their report, Pries et al. (2023) mean that exposure can lead to serious health effects, including respiratory irritation, headaches, nausea, and, in severe cases, pulmonary, neurological, and cardiovascular damage.

Furthermore, sargassum also has a negative impact on infrastructure due to the corrosive nature of hydrogen sulfide when it reacts with water (Pudi et al. 2022). Additionally, the corrosive nature of decomposing Sargassum accelerates the deterioration of boats, breakwaters, and other maritime infrastructure. Thick accumulations of the algae can also clog propellers and block cooling water intakes in coastal power plants, disrupting local economies and public utilities (Pries et al. 2023).

However, despite the problems associated with Sargassum, it has shown potential for utilization and carbon sequestration. Pries et al. (ibid.) reports that because of their high productivity and efficiency in trapping sediment-bound carbon, these algae are considered superior to many terrestrial plants in capturing and storing atmospheric carbon dioxide (CO_2). Although organic material produced through photosynthesis can follow various pathways, once it sinks to the ocean floor, the carbon it contains is effectively sequestered and prevented from exchanging with the atmosphere for several hundred to thousand years. So, when Sargassum biomass sinks—due to the collapse of its gas bladders under pressure—it carries organic carbon to the deep ocean, effectively removing it from the atmosphere. Globally, around 11% of macroalgal suspended organic carbon reaches the deep sea, serving as an important carbon sink (Pries et al. 2023).

2.3 Biogas Production from Sargassum

There are several potential commercial uses for macroalgae, including food, cosmetics, supplements, and fertilizers (ibid.). The increasing occurrence of Sargassum blooms in tropical and subtropical waters has sparked interest in its potential utilization for biofuel production. Currently, a majority of Sargassum removed from coastal areas is deposited in large landfills (Gray et al. 2021). Studies have shown that these landfills, along with the decaying Sargassum left on beaches, emit substantial amounts of methane, a potent greenhouse gas (ibid.). This methane potential, combined with the ongoing transition towards renewable energy systems and the global shift away from fossil fuels, has renewed interest in utilizing Sargassum as a feedstock for biogas production through AD. Historically, biogas production from seaweed was used as early as the nineteenth century due to the relative simplicity of the process in terms of engineering and infrastructure (Milledge and Harvey 2016b). In addition, Anaerobic Digestion (AD) offers the potential to exploit the full organic carbon content of macroalgae and is well suited to substrates with a high moisture content, without incurring significant energy penalties associated with dewatering or drying (ibid.).

A comprehensive understanding of the biogas potential of Sargassum requires insight into its chemical composition and constituent compounds. The ash content is relatively high at around 32-33% of dry matter (DM) , which reduce the proportion of fermentable organic matter and lower its overall energy content (Milledge and Harvey 2016a; Milledge and Harvey 2016b). The carbon content ranges from 28.9–30.1% and it has a high nitrogen fraction of 3.6–4.0% (Milledge and Harvey 2016a; Milledge and Harvey 2016b). Due to their high ash content, the higher heating values (HHV) of Sargassum species are around 12 $\mathrm{kJ \cdot g^{-1} DM}$, which is lower than those of terrestrial energy crops of 17–20 $\mathrm{kJ \cdot g^{-1} DM}$ (Milledge and Harvey 2016a; Milledge and Harvey 2016b). However, when adjusted for ash-free volatile solids, the HHV approaches around 18 $\mathrm{kJg^{-1}}$, comparable to that of carbohydrates at 17.2 $\mathrm{kJ \cdot g^{-1} DM}$ and proteins 21 $\mathrm{kJ \cdot g^{-1} DM}$ (Milledge and Harvey 2016b).

Studies has been conducted on biogas production from sargassum, both on the pelagic species, *Sargassum natans* and *Sargassum fluitans*, as well as the benthic *Sargasssum Muticum*. The biomethane potential of sargassum has been reported to be relatively low, with values between 0.06-0.14 $\mathrm{L_{CH_4} \cdot g_{VS}^{-1}}$ (Milledge and Harvey 2016b; Gray et al. 2021). The theoretical values presented are much higher at just under 0.4 $\mathrm{L_{CH_4} \cdot g_{VS}^{-1}}$ (Gray et al. 2021). Despite theoretical estimations indicating higher methane yields, practical results have consistently shown underperformance. The discrepancy between theoretical and experimental yields is not yet fully understood, and further research is needed to elucidate the limiting factors in AD (Milledge and Harvey 2016b; Gray et al. 2021).

Despite its potential as a substrate for AD, the use of Sargassum for biogas production presents several challenges. Sargassum are known to accumulate heavy metals from their environment, including concentrations of arsenic up to 231 $\mu\mathrm{g} \cdot \mathrm{g_{DW}^{-1}}$ (Milledge and Harvey 2016b). Such contamination not only raises environmental and health concerns, but also limits the potential use of digestate as fertilizer due to risks of soil accumulation. Additionally, sand in harvested biomass can cause operational issues and has been linked to reduced methane yields in pilot-scale systems (ibid.). Furthermore, high salt concentrations, commonly found in marine biomass, can inhibit methanogenic activity and reduced methane yields (Milledge and Harvey

2016a).

2.4 Biomass storage methods

One of the main limitations in developing a sustainable Sargassum-based value chain is its seasonal availability. Since harvesting tends to be irregular and seasonal, efficient preservation methods are needed that allow year-round supply to processes such as biogas production through AD (Milledge and Harvey 2016a; Gray et al. 2021). Preservation methods for macroalgae must address both biological stability and economic feasibility. The two main preservation methods currently investigated for Sargassum and other macroalgae are drying and ensiling (Milledge and Harvey 2016b). Each method presents distinct advantages and challenges, particularly in relation to energy consumption, compound degradation, and storage logistics.

2.4.1 Drying

One common preservation method is drying, it involves the removal of water from biomass. There are different drying methods presented by Milledge and Harvey (ibid.). The most common ways is either through solar exposure or thermal evaporation. Thermal evaporation uses heat that is transferred to the material, which make the moisture in the biomass evaporate and thereby dry (Neikov 2019). This process is notably energy-intensive, approximately 2.6 $MJ \cdot kg^{-1}$ is required to heat water from 20°C to 100°C and evaporate it at atmospheric pressure (Milledge and Harvey 2016b). Although mechanical dewatering prior to thermal drying has observed to reduce energy demand, the overall energy balance remains unfavorable. This makes large-scale thermal drying impractical and unsustainable from an environmental standpoint (ibid.).

The other method is trough solar exposure or Sun-drying, which is a more energy-efficient alternative, as it relies on natural solar radiation (Min et al. 2022). In tropical climates, drying may take 2–3 days under sunny conditions but can extend up to a week during rainy periods (Milledge and Harvey 2016b). Although inexpensive, solar drying requires significant land area and is heavily weather-dependent (Min et al. 2022). Additionally, it can cause substantial denaturation of organic compounds in the biomass, reducing its suitability for high-value applications. (Milledge and Harvey 2016b).

There is a third drying method, freeze-drying, that is more gentle on organic material, in contrast to both solar drying and thermal drying, which can cause damage to it (Milledge and Harvey 2016b; Min et al. 2022). However, it is economically infeasible for large-scale seaweed preservation. It is mostly limited to research applications or high-value products due to its high cost and energy consumption (Milledge and Harvey 2016b).

From a logistical standpoint, the drying process requires large open areas that are both flat and exposed to sufficient sunlight over extended periods. Given the scale of biomass that would need to be processed for a continuous biogas production, the spatial demand becomes a significant limitation, especially on small islands like Grenada where available land is limited (Milledge and Harvey 2016b; Min et al. 2022). In addition to land use, drying is time-consuming, and achieving the desired moisture content for storage can take up to a week depending on weather conditions. Moreover, studies have shown that solar drying can result in up to 15% loss of biomass due to physical degradation and organic matter volatilization during the drying process (Min et al. 2022), making the overall process less economically and energetically attractive.

Another key limitation is related to the rehydration requirements of dried biomass. AD processes require a high moisture content at minimum of 84% depending on substrate and digester type, for optimal microbial activity and substrate degradation (Ward et al. 2008). If the biomass is dried as a preservation method, it must later be rehydrated using fresh water to achieve the necessary moisture content for digestion. This poses a major challenge in Grenada, where freshwater availability alreday is limited (Thompson 2019). The country relies predominantly on surface water and rainwater harvesting, both of which are highly seasonal (Ministry of Climate Resilience 2025). During the rainy season, daily freshwater yields are estimated at 54,600 cubic meters, while they drop to only 31,800 cubic meters in the dry season. In contrast, demand increases during the dry season to 54,600 cubic meters per day, indicating a serious supply deficit over the year (ibid.). Forecasts indicate a 21% decrease in annual rainfall due to climate change, along with rising temperatures and more frequent drought events, limiting fresh water even more (ibid.). Saltwater intrusion from rising sea levels, reduced river flows from population growth and tourism, and pollution from agriculture further threaten the island's already fragile water system (Thompson 2019). These factors make large-scale use of freshwater for biogas substrate preparation—such as rehydrating dried Sargassum—an unsustainable solution. Therefore, while drying may seem attractive due to its low-energy input, the practical and environmental limitations associated with land use, biomass loss, and freshwater requirements pose significant barriers to its implementation as a large-scale storage method in Grenada.

2.4.2 Ensiling

Ensiling is a well-established method for the preservation of crops with high moisture content, traditionally employed in terrestrial agriculture for the storage of forage crops intended for animal feed (Milledge and Harvey 2016b). Given the high moisture content and rapid post-harvest degradation of seaweed biomass, ensiling is considered a promising alternative preservation strategy for macroalgae, including Sargassum species (Milledge and Harvey 2016a; Herrmann, FitzGerald, et al. 2015).

The fundamental mechanism of ensiling is based on anaerobic lactic acid fermentation. Under oxygen-deprived conditions, lactic acid bacteria (LAB), which can be either naturally present or supplemented as inoculants, metabolize water-soluble carbohydrates into organic acids, predominantly lactic acid (Herrmann, FitzGerald, et al. 2015; Milledge and Harvey 2016a; Milledge and Harvey 2016b). This acid accumulation leads to a decrease in pH, typically to values around 4.0–5.1 in Sargassum silage (Milledge and Harvey 2016a). The pH reduction inhibits the activity of spoilage microorganisms such as Clostridia, Enterobacteria, and yeasts (Herrmann, Heiermann, and Idler 2011; Milledge and Harvey 2016a). The optimal pH range for the proliferation of LAB is reported to be between 3.8 and 4.2 (Seydoşoğlu 2019) (De Man 1952). However, as seen in figure 3, the optimal pH is dependent on the moisture content in the biomass used. A rapid pH decline is critical as it promptly halts the wasteful activities of live plant material and undesirable microorganisms, thereby reducing losses. It also conserves more sugars for conversion to lactic acid, reducing the overall sugar requirement for successful fermentation (Ecosyl n.d.). A slow pH drop or sugar emptying before a stable pH is achieved can allow undesirable microorganisms, such as clostridia, to proliferate and convert lactic acid into the weaker butyric acid, leading to a detrimental increase in pH (clostridial secondary fermentation) (ibid.).

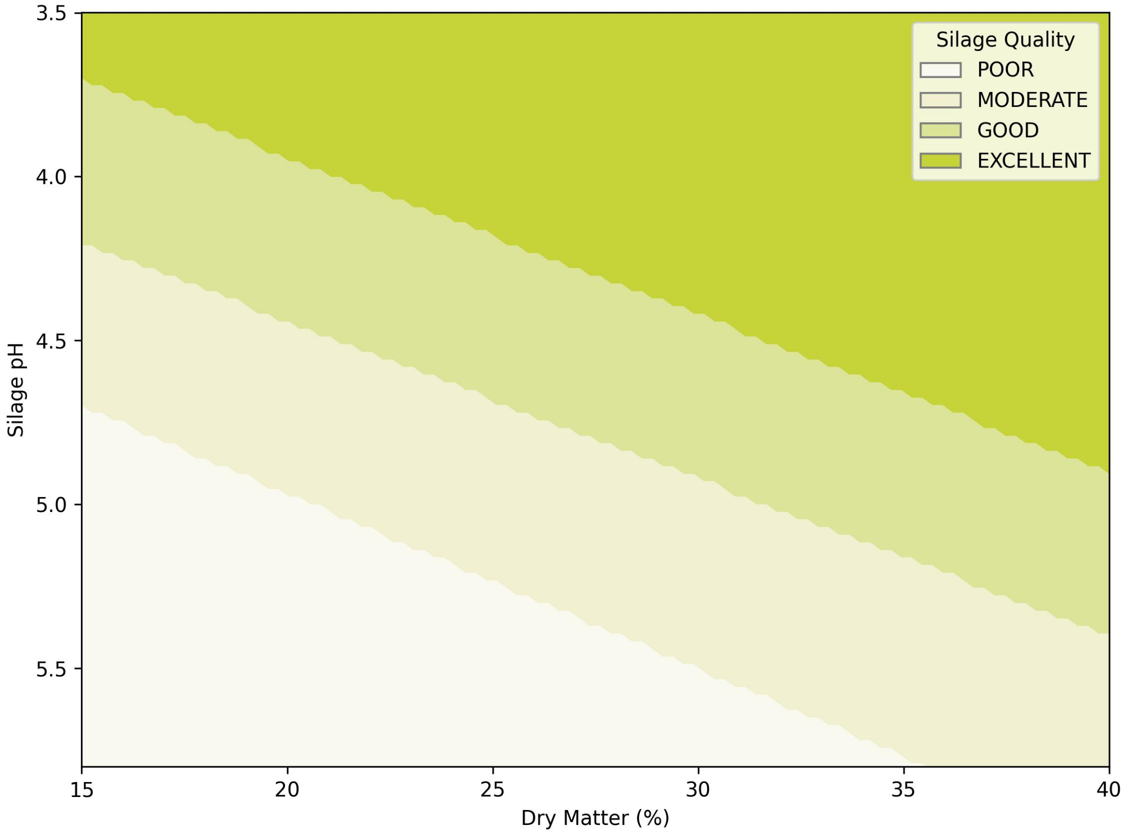

Figure 3: Relationship between silage pH and dry matter content, illustrating how preservation quality is influenced by initial moisture levels in the ensiled biomass (Ecosyl n.d.).

The ensiling process can be divided into four main phases (Herrmann, Heiermann, and Idler 2011; Borreani et al. 2018):

- Aerobic Phase: Immediately after sealing, residual oxygen is consumed through microbial activity and plant respiration.
- Anaerobic Phase: LAB dominate, producing lactic acid that rapidly lowers the pH. Under optimal conditions, this phase lasts only a few days and results in stable, high-quality silage.
- Fermentation subsides, and the biomass remains preserved if anaerobic conditions are maintained. This phase can last until the feed-out period. However, a slight but continuous fermentation may occur during an extended storage, leading to ongoing increases in fermentation products and storage losses.
- Feed-out Phase: The silage is exposed to air and used for downstream applications.

Although spontaneous fermentation may be sufficient, silage additives are used to obtain an efficient fermentation. Chemical additives normally inhibit or restrict undesirable silage fermentation or aerobic deterioration (Herrmann, Heiermann, and Idler 2011). These can include organic acids (e.g., formic acid, propionic acid, benzoic acid) and their salts (e.g., sodium benzoate, sodium propionate, sodium formate) (Jiang et al. 2024; Salinity 2025). Organic acids rapidly lower pH and reduce bacterial activity, also possessing bactericidal effects that promote the dominance of acid-tolerant LAB (Salinity 2025). However, acids are less effective against yeast, potentially leading to higher ethanol levels in the silage and thereby higher energy losses (Salinity 2025; Kung Jr. et al. 2018).

Microbial inoculants, such as LAB, can enhance the speed and extent of pH reduction (Herrmann, Heiermann, and Idler 2011). There are two different LAB, homofermentative and heterofermentative, that follows different path ways during fermentation. Generally homofermentative LAB are preferred over heterofermentative LAB, which produce a mix of lactic acid, acetic acid, ethanol, and CO2, resulting in higher DM losses (Herrmann, Heiermann, and Idler 2011; Borreani et al. 2018). The effectiveness of biological additives is most pronounced in crops with slow ensiling characteristics, while having less effect in easily ensiled crops like maize with high sugar and low buffering capacity (Salinity 2025).

While ensiling is well-established in land-based systems, its application to marine biomass remains relatively underexplored. Research into the preservation of seaweed through ensiling dates back to the 1950s (Milledge and Harvey 2016b), with more recent studies conducted in Ireland and the UK focusing on the benthic species Sargassum muticum (Milledge and Harvey 2016a). These studies have shown that ensiling is an energy-efficient method for storing seaweed intended for biofuel production, particularly for biogas via AD. Reported energy losses are low, under 8%, and methane yields remain largely unaffected by the preservation process (ibid.).

While the final pH values achieved during Sargassum ensiling at 4.9–5.1 are comparable to those found in commercial grass silage in temperate regions (4.5–5.8), they may be insufficient to completely inhibit clostridial fermentation (ibid.). This is primarily due to the high moisture content and low DM of seaweed relative to traditional terrestrial silage crops. As a result, butyric acid production may occur, potentially compromising silage quality and digestibility (ibid.).

To address the issue of low DM content, pre-ensiling treatments such as wilting, dewatering, or drying are often necessary (ibid.). The UK Ministry of Agriculture, Fisheries and Food (MAFF) recommends that forage be wilted to at least 25% dry matter prior to ensiling to minimize effluent production and environmental impact. Wilting also concentrates sugars, improving fermentation efficiency and reducing offensive odors (Milledge and Harvey 2016a; Borreani et al. 2018). However, only rapid wilting is beneficial, hence slower wilting may lead to biomass degradation and increased loss of organic material through microbial spoilage (Milledge and Harvey 2016a). Dry matter content above 35% offers protection against clostridia, but LAB activity may be reduced, potentially leading to less water-soluble carbohydrates in drier silage (Salinity 2025). Furthermore, a more rapid decrease in silage pH is observed in forages with low DM content under 30% compared to those with DM over 40%, because of more water-soluble carbohydrates (Kung Jr. et al. 2018). In addition, mechanical size reduction of the biomass through chopping or milling increases the surface area-to-volume ratio, facilitating microbial access to fermentable carbohydrates (Milledge and Harvey 2016a). Maceration prior to ensiling has been shown to increase fermentation rates, elevate lactic acid concentrations, and reduce ethanol formation. However, the specific effects of these treatments on seaweed ensiling remain under-researched (ibid.).

One of the key benefits of ensiling for biofuel applications is that the fermentation products are easily digestible, which can enhance methane yields in AD systems (Herrmann, FitzGerald, et al. 2015). These increased yields can offset or compensate for the minor energy losses incurred during storage (ibid.). Therefore, ensiling is considered particularly well suited for seaweed where biogas is the primary product. Losses associated with fermentation, primarily from carbon dioxide production, typically range from 2% to 4% (Zimmer 1980). Providing an effective seal on silos and silage piles is crucial to minimizing DM losses during the storage period, requiring a low-permeability barrier effectively secured to the crop and silo structure (Borreani et al. 2018).

3 Methods

This section outlines the methodology applied in this study. It includes the materials used, the experimental procedures conducted, and the analytical methods employed to evaluate the effects of different preservation techniques for Sargassum.

3.1 Algae Pre-treatment

Sargassum biomass was manually collected from the coastal waters near Soubise, Grenada. The algae were harvested directly from the sea to ensure freshness and avoid pre-degraded biomass from the shore. The biomass was immediately rinsed using clean seawater to remove sand and debris, and then transported in a seawater-filled bucket to prevent decomposition from freshwater exposure or desiccation in air.

The collected seaweed was chopped into fragments approximately 1-2 cm in size with a knife to ensure uniform mixing and representative sampling. The moisture content of freshly harvested biomass, was determined in a domestic microwave oven using the microwave drying method described in Bucholtz (2007). The samples were placed on a pre-weighed plate, and the combined weight of the plate and sample was recorded. The sample was then dried using a microwave oven on the highest setting. For the initial period of four minutes with the placement of a cup of water to prevent scorching.. Based on the initial DM measurements, two additional moisture levels were targeted through solar drying:

- 30% DM
- 50% DM

The biomass was spread in a thin layer on metal trays and left to dry under natural sunlight. Regular weighing of the material was performed during drying to monitor moisture loss. The process was terminated once the biomass reached the desired DM level.

3.2 Experimental setup

For the ensiling experiment, the pretreated biomass was divided into six treatment groups, each assigned to resealable 1 L PE plastic bags filled to capacity with the respective biomass. The six treatment groups were:

- Control group - raw biomass without additives (CTRL)
- Raw biomass with ensilage agent (RAW)
- Biomass dried to 30% DM with ensilage agent (30DM)
- Biomass dried to 50% DM with ensilage agent (50DM)
- Raw biomass with 2% sugar (sucrose) addition and ensilage agent (2S)
- Raw biomass with 5% sugar addition and ensilage agent (5S)

Notice that all treatment groups except for the control (CTRL) has an added ensilage agent. The ensiling agent used in the experiment was Ecosyl 50 from Volac, with the active lactic acid bacteria strain MTD/1. It was mixed with water to create a liquid solution according to the manufacturer's instructions of 2 L additive per metric ton biomass. The solution was applied with a 0.1 mL measuring cylinder and distributed evenly throughout each treatment group, except the control. After mixing, each bag was tightly sealed, minimizing air in the bag to reduce oxygen exposure.

All sealed bags were stored outdoors in a shaded and dark location to mimic real-world storage conditions under tropical ambient temperatures. Average daily temperatures during the storage period were approximately 27 °C, with an range between 25 to 29 °C This setup allowed observation of ensiling under conditions that approximate large-scale storage without artificial climate control.

3.3 Sampling and Analytical Methods

The experiment was divided into two parts, one for short time observation of pH changes with sugar as an additive, and one for a longer time testing pH, DM and volatile solids (VS) with different DM.

3.3.1 Short-term pH observation

To observe early acidification, which is critical for successful ensilage, pH measurements were taken every 6 hours during the first 72 hours on the four treatment groups:

- CTRL
- RAW
- 2S
- 5S

The sampling was performed by gently isolating a portion of the ensilage liquid from the biomass within the sealed bag. A small pocket was created with liquid separate from the biomass, creating a possibility to open the bag without air entering. This allowed insertion of the pH probe without introducing oxygen into the biomass. A digital pH meter and analog litmus paper were used in parallel to validate readings and detect any instrumentation drift.

3.3.2 Weekly Sampling for pH, DM, and VS

To study how ensilage as a storage method affects the sargassum biomass and its biogas potential, a longer observation period was chosen for four of the treatment groups:

- CTRL
- RAW
- 30DM
- 50DM

The four treatment groups were sampled once every seven days during an eight week period. For each sampling, approximately 30 g of biomass was extracted using clean instruments from each bag. To reduce oxygen contamination when sampling, a portion of the biomass was first gently separated inside the bag to isolate the sampling area, effectively reducing airflow into the rest of the biomass. After extraction, pH was immediately measured using the same method as described in section 3.3.1.

The biomass sample was then drained to remove any excess surface moisture adhering to it, and weighed to determine wet mass before DM and VS analysis. To determine DM determination, the biomass was dried using the microwave drying method. After this, the sample was weighed again and drying continued in two-minute intervals, with the weight recorded after each interval.

This process was repeated until the weight remained stable within ±1 gram, indicating that the sample was fully dried. The dry matter content was then calculated using equation 1.

$$\text{DM} = (\frac{m_{dry}}{m_{wet}}) * 100 \quad [\%] \tag{1}$$

Where DM stands for dry matter, and is returned in percentage, m_{dry} stands for the dry biomass weight after drying and m_{wet} stands for the wet biomass before drying.

VS analysis was conducted according to the methodology described by Herrmann, Heiermann, and Idler (2011) and Kreuger, Nges, and Björnsson (2011). The the dried biomass were ground using a mortar and pestle to reduce the particle size of the sample to a powder. The powder was placed on a metal tray and heated in a muffle furnace at 550 °C for 30 minutes. After cooling in a desiccator, the residual ash was weighed again using a scale with a precision 0,01 g. The VS as a percentage of DM was calculated using equation 2.

$$VS = (\frac{m_{dry} - m_{ash}}{m_{dry}}) * 100 \quad [\% \ of \ DM] \tag{2}$$

Where m_{dry} stands for the dry biomass weight after drying and m_{ash} stands for the remaining ash after the volatile compounds has evaporated.

Note, the study was conducted in two geographical locations, with the biomass being transported from the first place to the second. The first part was conducted on Grenada and the second part in Sweden. The dried samples were stored in a freezer before transport to minimize losses, but did not remain in a frozen state during the transport. The resealable bags with the ensiled samples were placed in an incubator upon arriving to Sweden, which was kept at 27°C. The weekly sampling was continued until the eight week period ended.

3.4 Data Analysis and Modeling

The dataset was compiled and analyzed statistically to assess trends in pH and VS content over time for the different ensilage treatment groups. For long-term stability assessment, a predictive model was developed in a Python script to extrapolate the evolution of volatile solids over a 6-month storage period.

The model was based on an exponential decay function in which the degradation rate was modulated by the observed pH values, serving as a proxy for the microbial activity. The equation used was

$$VS(t) = VS_0 * e^{-k*pH*t} + C \tag{3}$$

where, VS(t) represents the volatile solids at time t, VS_0 is the initial VS concentration, k is a decay constant describing the pH-dependent degradation rate, and c represents the non-degradable residual fraction. The pH at each time point was treated as an external input to the model.

Model parameters VS_0 k and C were fitted separately for each treatment group using non-linear least squares regression via the $curve_fit$ function from the SciPy library. The sum of squared errors between measured and predicted VS values over the 8-week period was minimized to determine the best-fit parameters. For extrapolation beyond the measured timeframe of

8 weeks, it was assumed that the pH values remained constant at their final observed value for each treatment group.

The model outputs were used to simulate and plot VS degradation over a 26-week period. Observed values and fitted curves were visualized using Matplotlib to allow comparison between actual measurements and predicted long-term behavior.

It is important to note that the extrapolated results extend beyond the calibration range of the model. As such, the predictions beyond week 8 should be interpreted as indicative trends rather than precise forecasts. The assumption of constant pH over the extrapolation period is a simplification, and actual microbial activity or environmental conditions may cause deviations from the predicted behavior.

4 Results

This section presents the results from the experimental evaluation of different storage and treatment methods for Sargassum. Key parameters analyzed include pH, Total solids (TS), and volatile solids (VS), with a focus on how these indicators evolved over both short-term and long-term ensiling periods. Each subsection corresponds to a specific part of the experimental design, allowing for a comparison between treatment groups and storage conditions. Detailed numerical data from the measurements are provided in table format in appendix A.

4.1 Short-Term pH Dynamics during Initial Ensiling

The short-term pH changes for the treatment groups: CTRL; RAW; 2S and 5S resulted in figure 4. During the first 72 hours, the pH value dropped in all groups, indicating a presence of acid-producing microbes. During the initial 10-12 hours the change in pH was limited for all groups, where CTRL, RAW and 2S all decreased slightly, but 5S increased to a pH of 6.6 from 6.5. After 12 hours all treatment groups showed a more rapid drop, with 5S decreasing the most from a pH of 6.5 to 4.6 in 36 hours. CTRL with untreated sargassum showed the shortest drop in pH. All test groups showed signs of approaching a plateau toward the end of the test period, as their values began to level out.

The results from the initial ensilage period suggests that Sargassum lacks a sufficient population of acid-producing microorganisms to induce a rapid pH decline, if ensiled without additives. The addition of lactic acid bacteria to the substrate significantly enhances acid production, as evidenced by the accelerated decrease in pH. Furthermore, the observed correlation between higher sugar concentrations and a more rapid pH decline, as well as stabilization at lower pH values within the optimal range for successful fermentation, indicates that Sargassum is inherently low in readily fermentable carbohydrates. These findings align with established knowledge of silage fermentation, where rapid acidification depends both on the presence of lactic acid bacteria and on an adequate supply of water-soluble carbohydrates (Milledge and Harvey 2016b; Herrmann, FitzGerald, et al. 2015).

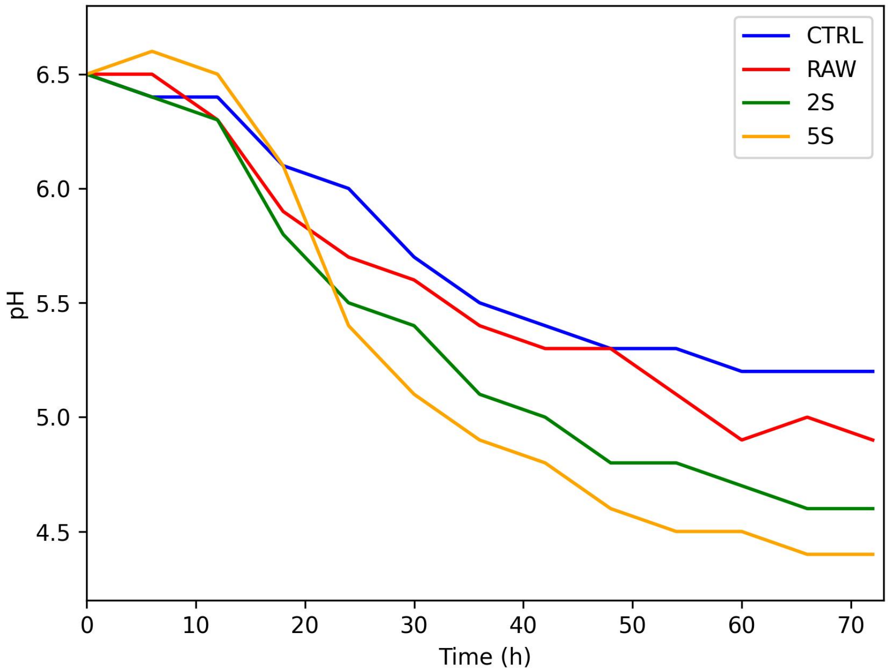

Figure 4: Change in pH during the first 72 hours after ensilage process for the treatment groups: Control group - raw biomass without additives (CTRL), raw biomass with ensilage agent (RAW), raw biomass with 2% sugar addition and ensilage agent (2S) and raw biomass with 5% sugar addition and ensilage agent (5S).

4.2 Long-Term Changes in TS, pH and VS

When comparing the changes in total solids (TS) content over the 8-week ensiling period for the treatment groups CTRL, RAW, 30DM, and 50DM, the following patterns were observed: The initial TS levels were approximately 16% for both CTRL and RAW, 30% for 30DM, and 50% for 50DM. Across all groups, a distinct drop in TS values was noted at week 2, diverging from the otherwise stable or increasing trends observed throughout the rest of the period (see Figure 5). This unexpected dip was most likely attributed to inconsistencies in the weighing process during the early stages of sample preparation prior to oven-drying for TS determination.

After this dip, all treatments gradually increased or stabilized in TS content, with the clearest upward trend observed in 50DM, which rose steadily from 50% to nearly 56% by week 8. 30DM initially dropped to around 24% at week 2, but recovered and was slightly above its starting value at approximately 31% week 8. In contrast, the fresh treatments (CTRL and RAW) remained consistently low, with the CTRL group reaching 20% and the RAW group just under 19% by week 8. This indicates that during the ensilage process the biomass slightly release some of its inbedded moisture, decreasing its moisture content.

The weekly pH change for the four treatment groups CTRL, RAW, 30DM and 50 DM, started at a uniform pH of 6.5. By week 1, all samples exhibited a sharp decrease, reflecting the onset of fermentation processes. In section 4.1 the change during the first days are shown. However, significant divergence in pH development became apparent from week 2 onward, which can be observed in figure 6. The treatment group 30DM showed the most stable and favorable trend, rapidly reaching a pH of 4.4 and maintaining it consistently throughout the 8 weeks. This suggests strong acid production and good fermentation stability.

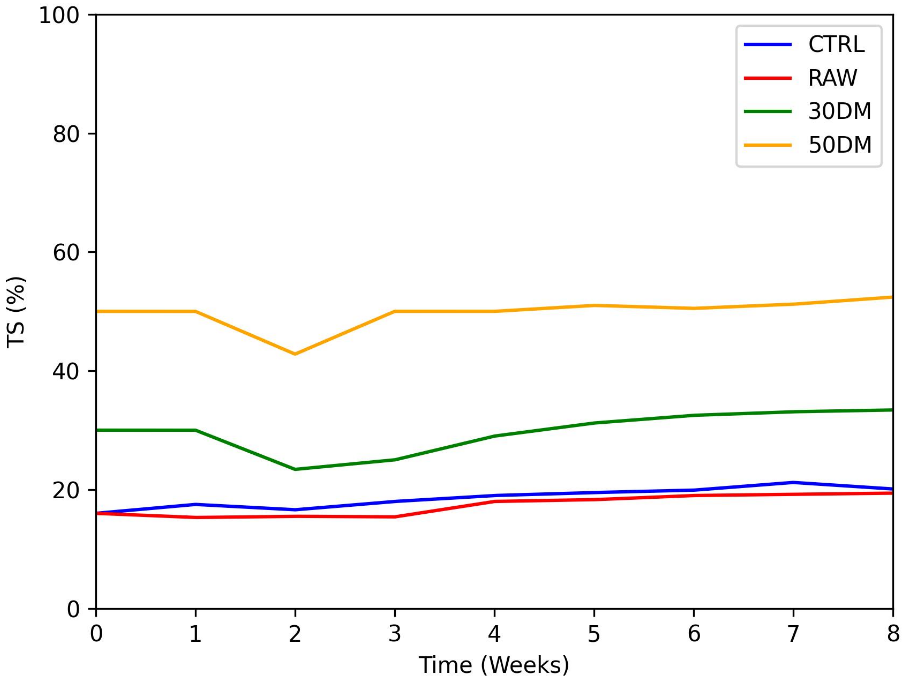

Figure 5: TS during a test period of 8 weeks. Testing the treatment groups: Control group - raw biomass without additives (CTRL), raw biomass with ensilage agent (RAW), biomass dried to 30% DM with ensilage agent (30DM) and biomass dried to 50% DM with ensilage agent (50DM).

50DM also quickly reached relatively low pH levels, but showed a gradual return to to approximately 4.9 toward the end, possibly indicating decreased fermentation or microbial inhibition due to higher TS content. In contrast, the fresh treatments (CTRL and RAW) exhibited progressive pH increase throughout the storage period. The CTRL group had the highest end value (pH 5.8), while the RAW group stabilized around a pH of 5.1. These elevated pH values indicate weaker acidification and a greater risk of spoilage or undesirable microbial growth. A common pattern is an initial pH drop for all treatments, but a TS increase clearly improves long-term acid stability, especially at the 30% TS level.

Finally, the change in VS for the four treatment groups over 8 weeks all started at a VS of approximately 60% before ensilage. Across all treatment groups, a decline in VS over time was observed, reflecting degradation of organic material during storage, seen in figure 7. The sharpest and most continuous decline occurred in the CTRL group, dropping to 37% by week 8. the RAW group followed a similar trend, but with a lower desegregation ending at a VS of 43%.

Treatment groups with higher TS showed better retention of organic matter. The 30DM group showed the slowest rate of VS loss, finishing at 49%. The 50DM group ended at 46% suggesting a slightly higher degradation than at the 30% TS level. All treatments shared the same general trend of decreasing VS content, but the rate and extent of this decline was clearly mitigated by higher TS levels. This suggests that dry matter supplementation effectively decreases microbial degradation of organic material, improving the conservation quality of the silage (figure 7.

When considered together, the result seen in figure 5, 6 and 7 for TS, pH and VS provides a consistent picture of how dry matter supplementation influences ensiling outcomes for Sargassum. The 30DM treatment stands out across all metrics: it shows stable or slightly increasing TS

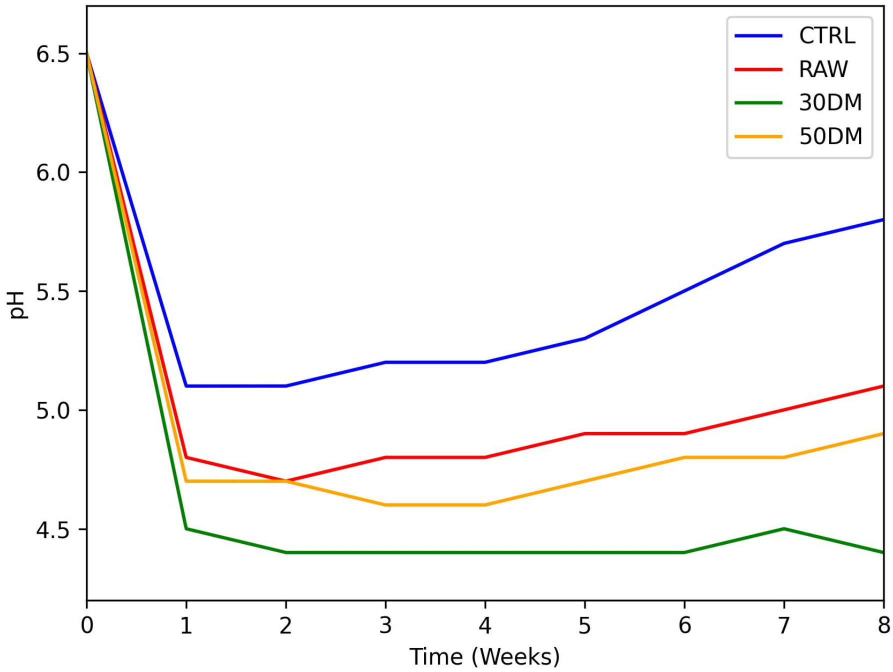

Figure 6: pH during a test period of 8 weeks. Testing the treatment groups: Control group - raw biomass without additives (CTRL), raw biomass with ensilage agent (RAW), biomass dried to 30% DM with ensilage agent (30DM) and biomass dried to 50% DM with ensilage agent (50DM).

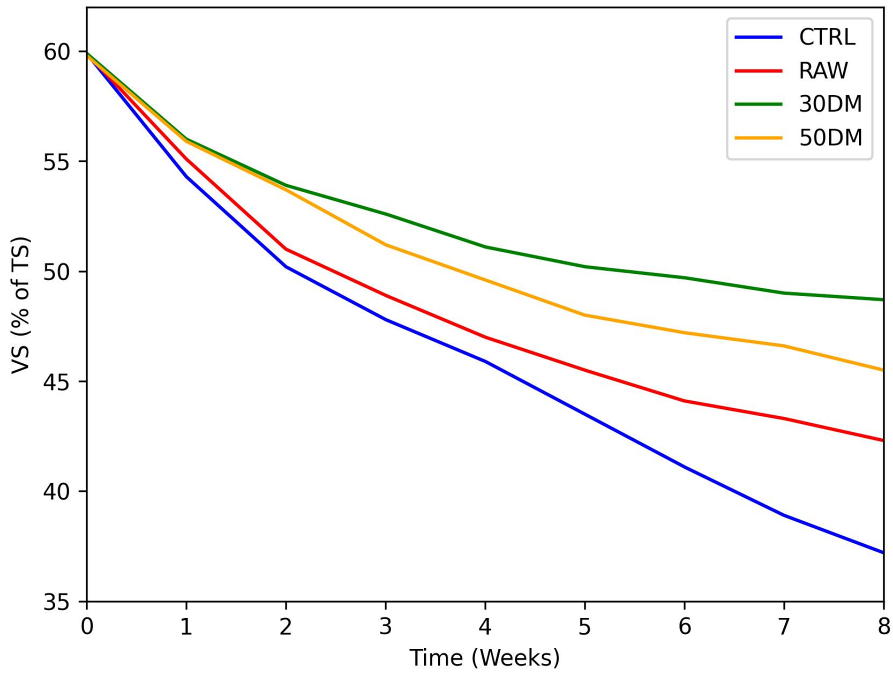

Figure 7: VS during a test period of 8 weeks. Testing the treatment groups: Control group - raw biomass without additives (CTRL), raw biomass with ensilage agent (RAW), biomass dried to 30% DM with ensilage agent (30DM) and biomass dried to 50% DM with ensilage agent (50DM).

content, consistently low and favorable pH, and the best preservation of volatile solids. While 50DM also performs well, its slightly elevated pH and greater VS loss suggest that to high TS content may limit fermentation efficiency, possibly due to suboptimal microbial activity or a limitation of carbohydrate solubility required for microbes.

The fresh treatments (CTRL and RAW) demonstrated significantly weaker performance. Both exhibited low and stagnant TS levels, rising pH, indicating poor fermentation, and a severe loss of organic material over time. These findings highlight the challenges of storing fresh Sargassum without adding acid-producing microorganisms and increasing sugar concentration. Overall, the results suggest that targeting a TS content of approximately 30% generates a favorable concentration of fermentable carbohydrates, while also retaining sufficient moisture to support the metabolic activity of lactic acid bacteria and other fermentative microbes. This combination promotes a stable and efficient fermentation process, leading to better pH control and reduced loss of organic material. As such, a TS level around 30% appears to offer an optimal balance between fermentation stability and organic matter preservation, making it a promising storage conditions for Sargassum intended for biogas applications.

Furthermore, the results indicate a correlation between pH value and VS content in the studied sargassum biomass. A better fermentation process that leads to a rapid pH decrease could lead to a quick stabilization of the VS value.

4.3 Modelling of VS Loss during Extended Storage

The predictive model estimates VS degradation over a six month period (26 weeks) for the four different treatments groups of Sargassum as in section 4.2 (CTRL, RAW, 30DM and 50DM). The VS of all treatment groups was initially approximately 60%, but the trends diverged significantly over time. A common pattern across all treatments was a rapid initial decline in VS values, followed by a gradual leveling off. This suggests that the easily degradable organic matter was consumed early in the storage period. The trend followed an exponential decay trend. The Model is displayed in figure 8.

From the fitted model it can be seen that the CTRL group undergoes significantly higher degradation of VS over time compared to the treated groups. As the storage period progresses, the differences in remaining VS between treatments become increasingly pronounced, indicating that the protective effects of the ensiling agents and increased TS content are not only immediate but also cumulative. The results highlight how the CTRL group's rapid decline contrasts with the more stable trajectories of the treated groups RAW, 30DM and 50 DM (figure 8). Among these, the 30DM treatment consistently exhibited the most favorable balance between long-term preservation and practical feasibility, suggesting it is a suitable and effective storage strategy for Sargassum intended for bioenergy applications.

As mentioned, the CTRL group exhibits the fastest VS reduction rate, reaching a final VS content just below 33% by week 26. This indicates high microbial activity and extensive degradation in the absence of any stabilizing treatment. The fresh biomass with silage agent (RAW) also showed a substantial reduction in VS, but the decline was less pronounced, stabilizing around 41%. This uggests that the addition of lactic acid bacteria helps the preservation of sargassum, reducing the presence of microbes that leads to biomass degradation.

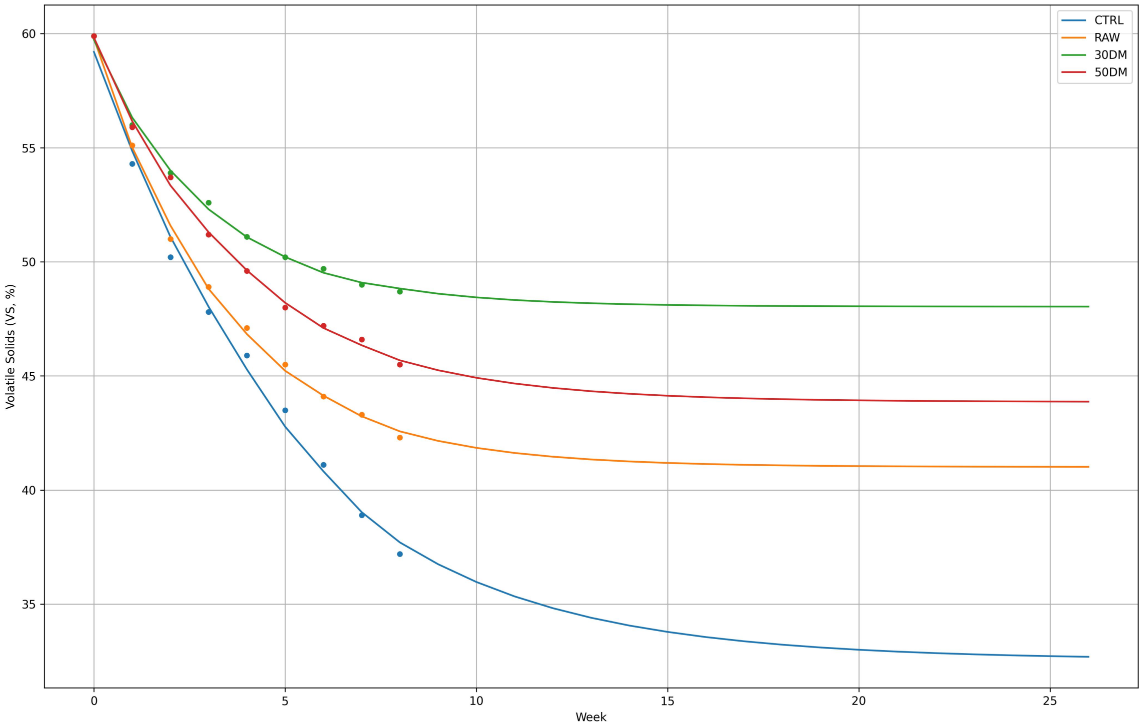

Figure 8: Estimated VS degradation over a six month period for treatment groups: Control group - raw biomass without additives (CTRL), raw biomass with ensilage agent (RAW), biomass dried to 30% DM with ensilage agent (30DM) and biomass dried to 50% DM with ensilage agent (50DM).

Treatments with elevated dry matter content (30DM and 50DM) demonstrated considerably slower degradation rates. The 30DM group leveled off at approximately 48% VS, while the 50DM group reached slightly lower values at around 44%. Despite the higher initial dry matter in the 50DM group, it did not appear to provide a proportionally greater stabilization effect over time. This may imply that there is a threshold beyond which additional dry matter yields diminishing returns in terms of preservation, or that 50DM do not provide a favorable climate for lactic acid bacteria to create a good preservation environment.

5 Discussion

The results of this study indicate that Sargassum, when ensiled, needs additives or drying to be effectively preserved for biogas production. This is observed in the insufficient and delayed drop in pH, the failure to reach a pH level low enough, and the overall lack of stabilization in pH over the storage period. Such deficiencies in the fermentation process most probably led to undesirable microbial activity, reducing the quality and energy potential of the preserved biomass. This is also confirmed in the results for VS, where the untreated Sargassum showed more losses of volatile solids compared to the treated groups, which is seen in figure 7. This indicates a greater organic matter degradation and energy loss during storage. In contrast, the treatment group with 30% DM shows signs of stabilization, as seen in the graphical representations of both pH in figure 6 and VS in figure 7 across the eight week storage period. This group appears to approach a plateau, especially in terms of pH, indicating a more controlled fermentation process and better preservation of organic matter. The other test groups, particularly the one without additives, fail to display the same level of stability, reinforcing the conclusion that both additives and a careful moisture to sugar management are critical for successful ensiling of Sargassum.

Moreover, the presence of water-soluble carbohydrates plays a key role in ensuring effective lactic acid fermentation. Increasing water-soluble carbohydrates appears beneficial, as it facilitates a quicker drop in pH and promotes the dominance of lactic acid bacteria. However, excessive drying can suppress microbial activity, underscoring the importance of maintaining a balance between dry matter and available sugars. The result is in line with previous studies that indicated that a dry matter content of 25-30% is optimal for lactic acid production in ensilage (Milledge and Harvey 2016a; Salinity 2025). One promising solution to increase the sugar concentration other than drying is the co-ensiling of Sargassum with sugar-rich substrates. On Grenada, suitable co-substrates may include molasses, sugarcane bagasse, or fruit and juice industry residues such as mango skins or banana waste. These materials could enrich the fermentable sugar content and improve the ensiling outcome, while also contributing to local circular bioeconomy practices.

Based on Grenada's commitment to processing 10,000 tons of Sargassum annually by 2026, the potential biogas production can be estimated using the experimental results from this study (The New Today 2025). Assuming that the preserved biomass reaches a volatile solids (VS) content of approximately 48% after ensiling (as observed in the 30% TS treatment), the total amount of usable organic matter is 4,800 tons. Using the reported methane yields ranging between 0.06 and 0.14 $\mathrm{L_{CH_4}} \cdot \mathrm{g_{VS}^{-1}}$, this corresponds to a yearly methane production of 288-672 $m^3\mathrm{CH_4}$. The energy content in methane gas is about 10 kWh$/m^3$ and gives an estimate of 2.9-6.7 MWh produced energy (Energigas Sverige 2023).

Despite these promising findings, the study has several uncertainties and limitations that must be acknowledged. Firstly, the limited access to precision instruments in Grenada introduced a number of measurement uncertainties. For instance, the scale used during the experiment had a precision of ±1 gram, which can significantly impact calculations based on small sample weights and lead to errors in percentage-based results. Additionally, TS was determined using a microwave drying method. While this method is mentioned in literature and has been used under field conditions, it is not a standardized or highly accurate scientific method. However, due to the lack of access to drying ovens or scales, it was considered the most practical option. Furthermore, the determination of VS poses another area of uncertainty. Ensiling produces various volatile compounds, including organic acids and alcohols, some of which may evaporate during the drying process used to measure TS. This is also true when using the standardized oven-method (Milledge and Harvey 2016a; Kreuger, Nges, and Björnsson 2011) This can lead to an underestimation of the actual organic content available for biogas production. For further studies it is recommended to analyze the concentration of produced acids and alcohols to correct the estimation of VS using the method presented by Porter and Murray (2001). The transport of samples from Grenada to Sweden for VS analysis further introduces risk. Since the samples were not frozen during air transport, microbial and enzymatic degradation may have continued leading to lower VS readings.

The results indicate that the volatile solids in ensiled Sargassum, crucial for biogas production, are projected to decrease by only approximately 12% over a six-month storage period. This minimal degradation is highly significant, as the seasonal and often unpredictable nature of Sargassum influxes require an effective preservation strategy. Ensiling thus emerges as a critical method for establishing a consistent, year-round feedstock supply for AD facilities, ensuring continuous biogas production irrespective of Sargassum availability. Furthermore, this preservation capability enables the efficient management of peak biomass inflows that often exceed immediate AD capacity. By ensiling surplus Sargassum instead of resorting to costly landfill disposal or leaving it to decompose on beaches, a greater proportion of the biomass can be valorized for energy production. This approach not only prevents the negative environmental and health impacts associated with uncontrolled decomposition but also yields substantial economic benefits for regions like Grenada by transforming a waste product into a valuable energy resource.

Today, Grenada uses only a small amount of solar and wind energy for its power supply (U.S. Department of Energy 2020). However, because of its location near the equator, the island nation has great and steady potential for solar energy throughout the year. For example, areas like the southern part of the main island, Carriacou, and Petite Martinique receive about 2.1 MWh of solar radiation per square meter each year (Solargis 2021). Due to its intermittent nature, solar power in Grenada is limited to producing electricity during the 12-13 hours of daily sunlight (Time and Date 2025). This requires large and often expensive systems to store electricity if continuous power during both day and night is needed. Therefore, energy sources that can be regulated depending on load or that can serve as a base-load, are essential to work alongside solar energy and keep the grid stable, especially when the sun is not shining. In this context, ensuring biogas production from Sargassum year around becomes an important and useful option. It offers a reliable and controllable energy output that can help balance the changing amount of solar energy and contribute to providing power all day around.

Moreover, the nature of sargassum as feedstock and the production process could facilitate decentralized energy generation, supporting local energy solutions and enhancing resilience across the island. This can be favorable for countries that prone to hurricanes or other weather phenomenons that can destroy large scale energy infrastructure. The establishment of this new energy sector would also create employment opportunities across the value chain, from Sargassum collection and pre-treatment to plant operation and digestate management, contributing to local economic development.

6 Conclusions

The findings demonstrate that untreated Sargassum (CTRL) is highly prone to degradation, with the steepest decline in volatile solids (VS) and the least effective pH stabilization over both short- and long-term storage periods. This confirms that ensiling conditions—particularly DM content and additive use—have a significant influence on preservation outcomes. The treatment with 30% dry matter (30DM) showed the most favorable characteristics: consistently low pH values, minimal loss of VS, and stable TS levels.

Short-term pH observations revealed that the addition of sugar and lactic acid bacteria accelerates acidification during the initial 72 hours. Especially the group with 5% added sugar (5S) achieved a rapid pH decline to 4.6 within 36 hours, emphasizing the importance of fermentable substrates in supporting microbial acid production.

Finally, an exponential model was developed to predict VS degradation over a six-month period. This model indicates that preservation benefits accumulate over time. The 30DM group demonstrated superior long-term stability compared to all other groups, with projected VS losses significantly lower the the CTRL group. The model also showed increasing divergence between treatments over time, reinforcing the conclusion that initial treatment decisions have lasting consequences for biomass integrity

7 Future research

Based on the findings and limitations of this study, several areas for future research have been identified that could further support the development and implementation of Sargassum-based biogas systems:

- **Pilot-scale validation:** Future studies should validate the most promising treatment—30% dry matter with silage agent—under real-world conditions, including outdoor storage, fluctuating temperatures, and practical handling constraints. Such pilot-scale trials would not only assess the method's scalability and robustness, but also provide valuable data to confirm or refine the predictive model developed in this study.
- **Biogas yield analysis:** Although preservation parameters were studied, the actual methane potential of the preserved biomass was not measured. Direct biogas production trials using ensiled Sargassum would offer critical insight into energy yield and process efficiency.
- **Microbial community profiling:** Exploring how microbial communities develop during ensiling under different treatments could clarify the mechanisms behind successful fermentation and spoilage, particularly in marine biomass systems.
- **Co-ensilage:** Research into co-ensilage of Sargassum with other organic waste streams (e.g., food waste, manure) may reveal synergistic effects that improve acid production, nutrient balance, or process stability.

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A Numerical data from measurements

Below are the numerical measurements from the experiment presented in tables. Each table is represents the values shown in figure 4 to 7 resented in the result.

Table 1: pH-value over a 72 h for short-term experiment presented in figure 4.

Tid (h)	CTRL	RAW	2S	5S
0	6.5	6.5	6.5	6.5
6	6.4	6.5	6.4	6.6
12	6.4	6.3	6.3	6.5
18	6.1	5.9	5.8	6.1
24	6.0	5.7	5.5	5.4
30	5.7	5.6	5.4	5.1
36	5.5	5.4	5.1	4.9
42	5.4	5.3	5.0	4.8
48	5.3	5.3	4.8	4.6
54	5.3	5.1	4.8	4.5
60	5.2	4.9	4.7	4.5
66	5.2	5.0	4.6	4.4
72	5.2	4.9	4.6	4.4

Table 2: pH-value over 8 weeks for long-term experiment presented in figure 6.

Tid (veckor)	CTRL	RAW	30DM	50DM
0	6.5	6.5	6.5	6.5
1	5.1	4.8	4.5	4.7
2	5.1	4.7	4.4	4.7
3	5.2	4.8	4.4	4.6
4	5.2	4.8	4.4	4.6
5	5.3	4.9	4.4	4.7
6	5.5	4.9	4.4	4.8
7	5.7	5.0	4.5	4.8
8	5.8	5.1	4.4	4.9

Table 3: TS over a 8 week period for the long-term experiment presented in figure 5 (value in %)

Tid (veckor)	CTRL	RAW	30DM	50DM
0	16.0	16.0	30.0	50.0
1	17.5	15.3	30.0	50.0
2	16.6	15.5	23.4	42.8
3	18.0	15.4	25.0	50.0
4	19.0	18.0	29.0	50.0
5	19.5	18.3	31.2	51.0
6	19.9	19.0	32.5	50.5
7	21.2	19.2	33.1	51.2
8	20.1	19.4	33.4	52.4

Table 4: VS over a 8 week period for the long-term experiment presented in figure 7 (value in %)

Tid (veckor)	CTRL	RAW	30DM	50DM
0	59.9	59.9	59.9	59.8
1	54.3	55.1	56.0	55.9
2	50.2	51.0	53.9	53.7
3	47.8	48.9	52.6	51.2
4	45.9	47.0	51.1	49.6
5	43.5	45.5	50.2	48.0
6	41.1	44.1	49.7	47.2
7	38.9	43.3	49.0	46.6
8	37.2	42.3	48.7	45.5

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