Mexican Journal of Technology and Engineering *Volumen 2, Número 1*

Artículo de investigación https://doi.org/10.61767/mjte.002.1.4452

Méndez-González et al., 2023

Recibido: 20-03-2023 *Revisado: 15-04-2023* *Aceptado: 28-04-2023* *Publicado: 30-04-2023*

THE SALT CONTENT IS NOT A LIMITATION FOR ENSILING SARGASSUM COLLECTED IN THE MEXICAN CARIBBEAN

EL CONTENIDO DE SALES NO ES UNA LIMITANTE PARA ENSILAR EL SARGAZO RECOLECTADO EN EL CARIBE MEXICANO

F. Méndez-González[1,*], B. Hernández-Zarco[2], and E. Favela-Torres[3]

[1] Laboratorio de Biotecnología y Bioingeniería, Centro de Investigación en Alimentación y Desarrollo A.C., Coordinación Delicias, C.P. 33089, Chihuahua, México.
[2] Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana, Unidad Xochimilco, 04960, México.
[3] Departamento de Biotecnología, Universidad Autónoma Metropolitana, Unidad Iztapalapa, 09340, México.
[*] Corresponding author: mg.fernandomendez@gmail.com

Abstract

Since 2011, sargassum has been infesting the coasts of the Mexican Caribbean, affecting the environment, human health, and economic activities. However, sargassum can be used via different processes to obtain alginates, fucoidans, biofuels, agricultural fertilizers, and livestock feed. Nevertheless, the quantity and composition of the sargassum that reaches the coasts of the Mexican Caribbean present a high seasonal variation, which hinders its industrial exploitation. Therefore, to ensure a supply of sargassum susceptible to industrial processing, the pretreatment conditions for its conservation through ensiling were determined. Among them, inoculum addition, sargassum desalination, and the incorporation of additives were evaluated. In sargassum without pretreatment, inoculum addition cannot effectively lower the pH in the silo. In contrast, glucose addition (~261 mg/gdm) in washed sargassum enriched with nutrient solution allows a pH decrease up to ~4.8, reaching an average glucose bioconversion to lactic acid above 68 %. Similar results were obtained when ensiling sargassum without washing, reaching the maximum decrease in pH after 10 d. In addition, low contents of acetic acid (< 20 mg/gdm) and ethanol (< 8 mg/gdm) were registered in these silos, indicating a low activity of other microorganisms. Therefore, adequate sargassum silage requires the addition of inoculant, glucose, and nutrient.

Artículo de investigación

Méndez-González et al., 2023

Keywords: *Lactobacillus casei*, lactic fermentation, silage additives, sargassum management, solid-state fermentation.

Resumen

Desde el 2011, el sargazo infesta las costas del Caribe Mexicano, afectando al medio ambiente, la salud humana y la economía. Sin embargo, el sargazo puede ser aprovechado para la obtención de alginatos, fucoidanos, biocombustibles, fertilizantes y alimento pecuario. No obstante, la cantidad y composición del sargazo que arriba a la costa presenta alta variación estacional; lo cual, dificulta su explotación industrial. Por lo tanto, para asegurar el suministro de sargazo susceptible de procesarse industrialmente, se determinaron las condiciones de pretratamiento para su conservación por ensilaje. Entre ellas, se evaluó la desalinización e incorporación de aditivos. La adición de inóculo no fue efectiva para disminuir el pH del sargazo sin pretratamiento. En contraste, adicionando glucosa (~261 mg/gms) al sargazo lavado y enriquecido con nutrientes, se disminuye el pH hasta ~4.8, alcanzando una bioconversión de glucosa a ácido láctico superior al 68 %. Resultados similares se obtuvieron ensilando sargazo sin lavar, alcanzando una máxima disminución de pH después de 10 d. Además, en ambos se registró bajo contenido de ácido acético (< 20 mg/gms) y etanol (< 8 mg/gms), indicando baja actividad de otros microorganismos. Por lo tanto, para ensilar el sargazo se requiere la adición de inóculo, glucosa y nutrientes.

Palabras clave: *Lactobacillus casei*, fermentación láctica, aditivos de ensilaje, manejo del sargazo, fermentación en estado sólido.

1. Introduction

Sargassum is a marine alga included in the division Phaoephyta [1] and whose population is concentrated in the Sargasso Sea [2]. From that region, small sargassum swarms arrive on the coast of the American and African continents. However, since 2011, sargassum has been accumulating and proliferating in the Great Atlantic Sargassum Belt [2]. This region concentrates approximately 2×10^7 t of algal mass and extends from the coasts of Brazil to the United States [3]. From the Great Atlantic Sargassum Belt, sargassum periodically arrives to infest the coast, with harmful effects on the environment [4], human health, and economic activity [5]. Whitin the infestation area is the Mexican Caribbean, which, from 2018 to 2019, reached a monthly sargassum arrival greater than 1.7×10^3 m^3 per km of coastline [5]. In Mexican Caribbean, the efforts aimed at sargassum removal and final disposal have been unsuccessful and expensive [6,7]. Therefore, to mitigate the damage and costs caused by sargassum infestation, different strategies have been developed for sargassum valorization. Among them are the extraction of alginates and fucoidans [8,9], bioconversion to ethanol and methane [1,10,11], and the incorporation into formulations of agricultural fertilizers [12–15] and livestock feed [16–18]. However, the quantity and composition of the sargassum arriving in the Mexican Caribbean present high seasonal variability, making its collection and viable disposal difficult [6]. This irregular sargassum supply is a great challenge for its industrial valorization. Therefore, it is necessary to establish methods for biomass conservation. One alternative could be ensiling, which is a simple, low-cost, and useful method for organic matter conservation under different climatic conditions [19]. Among the sargassum properties that make ensiling difficult are the associated microflora

[20], high salinity [21], and low fermentable sugar content [22]. Therefore, it is necessary to develop a pretreatment method that allows the ensiling of sargassum. Implementing ensiling as a sargassum conservation method could contribute to its industrial exploitation.

2. Materials and methods

2.1 Sargassum and silos

Sargassum biomass was collected (August 2019) on the coast of Puerto Morelos (Mexico), dried (up to ~8 %), cut into 1-cm-long pieces, and stored in sealed plastic bags. Falcon tubes (50 mL) with a capacity of ~40 g of wet sargassum (moisture content ~80 %) were used for sargassum ensiling.

2.2 Selection of the moisture content of the sargassum bed

The initial moisture content of the sargassum bed was selected by its water activity (a_w). Sargassum samples were prepared with different moisture contents (8 %, 50 %, 80 %, and 92 %). The water activity (a_w) was measured in three samples (5 g) for each moisture level with the AquaLab equipment (CX-2).

2.3 Inoculant addition effect

The inoculant effect was evaluated by the addition of *Lactobacillus casei*. Silos were packed with moist sargassum (moisture content 80 % and pH 7.4) without inoculation and inoculated with ~1 × 10^6 CFU per gram of dry sargassum (gds) (four silos per treatment). The silos were incubated in the darkness at 30°C. After 20 d, the pH of the fermented material in each silo was measured in duplicate.

2.4 Sargassum washing

To determine the process for washing sargassum, samples of ~5 gds were placed in 500-mL beakers, and 200 mL of deionized water (pH = 5.99 and EC = 148.90 µS/cm) was added to each sample and stirred with a magnetic bar for 10 min. Subsequently, the water was decanted, and the sargassum was drained and washed again (up to completing five washes). At the end of each washing cycle, the pH and electrical conductivity (EC) in the residual water were measured.

2.5 Effect of the carbon source addition on the silage of washed sargassum beds enriched with nutrients

Glucose and nutrient solution (NS) were used as additives. NS was composed of Casein Peptone (10 g/L), Meat Extract (8 g/L), Yeast Extract (4 g/L), Magnesium Sulfate (0.2 g/L), Manganese Sulfate (0.2 g/ L). To evaluate the effect of glucose addition, NS was prepared with different glucose concentrations (20, 50, and 100 g/L). The three medium configurations were used to impregnate washed and dried sargassum up to 80 % w/w for prepared silos with 1.6 %, 4 %, and 8 % glucose (wet basis). Subsequently, the impregnated sargassum was inoculated with ~1 × 10^6 CFU of *L. casei*/gds and packed in the silos, followed by incubation in the dark at 30°C. At 3, 7, 10, and 14 days of incubation, silos were taken from each treatment for the determination of electrical conductivity, pH, and lactic acid production in the fermented mass.

2.6 Silage process sensitivity to washing and nutrient addition

To determine the effects of sargassum washing and nutrient addition, a 2 × 2 factorial design was implemented (Table 1); the evaluated factors were washed (WS) and unwashed (US) sargassum and addition of distilled water (W) or nutrient solution (NS). NS were supplemented with 10 % glucose (w/v) and added to the sargassum up to 80 % w/w. All treatments were inoculated with *L. casei* (~1 × 10^6 CFU/gds) and packed in silos for incubation (at 30°C). At 3, 7, 10, 14, 17, and 21 days, silos were taken from each treatment for the measurement of pH as well as lactic acid (LA), acetic acid (AA), and ethanol (Et) production in the fermented mass.

2.7 Analytical methods

The pH, EC, and the contents of LA, AA, and Et were measured in suspensions of 1 gram of sample (wet mass) in 10 mL of distilled water. For that, the suspension was stirred in a vortex for 30 s. The pH and EC were measured in the

supernatant with a potentiometer (HANNA Instruments, HI5522). Subsequently, the suspension was filtered and centrifuged. Finally, the contents of LA, AA, and Et were determined with a high-performance liquid chromatography (HPLC) system (Perkin Elmer LC-250), using an isocratic method. An Aminex HPX-87H ion-exclusion column (BioRad, Hercules, CA, USA) eluted with 5 mM H_2SO_4 solution supply at 0.6 mL/min and 40°C was used, and the metabolites were detected with a refractive index (IR) detector (Perkin Elmer LC-30).

2.8 Statistical analysis

Continuous variables related to nominal variables were analyzed with the Shapiro-Wilk goodness of fit test. Datasets with normal distribution were analyzed with ANOVA ($\alpha = 0.05$), whereas those that did not correspond to normal distribution were analyzed with the Kruskal-Wallis's test. Subsequently, datasets with significant differences were analyzed by Tukey´s test.

Table 1. Silage treatments with and without washing and nutrient solution addition.

Treatment	Washing	Impregnation medium
USW	No	Water
USNS	No	Nutrient solution
WSW	Yes	Water
WSNS	Yes	Nutrient solution

Table 2. Sargassum water activity with different moisture content.

Moisture content (%)	Water activity
8.3	0.32 ± 0.01
50	0.84 ± 0.07
80	0.90 ± 0.05
92.4	0.95 ± 0.01

3. Results and discussion

To determine the pretreatment to sargassum silage, inoculant addition, sargassum desalination, and additive incorporation (carbon and nutrient source) were evaluated. Once the initial ensiling conditions were determined, the effects of removing the sargassum desalination and nutrient addition were evaluated to make the process more efficient.

3.1 Selection of the moisture content of the sargassum bed

The water availability in the silage bed is essential for lactic acid bacterial growth and biological activity [23]. The water availability in sargassum samples with different moisture contents was determined by its a_w. Sargassum samples with moisture contents from 80 % to 92 % presented an a_w from 0.90 to 0.95 (Table 2). Therefore, to avoid water limitations, sargassum must be moistened to at least 80 %. However, a high moisture content could limit fermentable sugar availability [24], reducing the production of AL and AA in the silo and increasing the possibility of the propagation of microorganisms that affect ensiling [23]. This makes it necessary to implement strategies that promote an effective pH decrease. Among them, inoculant addition will be evaluated in the next section.

3.2 Inoculant addition effect

The effect of adding *Lactobacillus casei* was evaluated in sargassum silos with a moisture content of 80 % and an initial pH of 7.4. After

ensiling for 20 d, the pH values of sargassum with and without inoculant were similar (ANOVA, α = 0.05), reaching values of 6.807 ± 0.16 and 6.968 ± 0.30, respectively. In both treatments, the pH tended to be neutral and was not suitable for sargassum conservation via ensiling. In crops and forage silages, inoculant addition (from 1×10^5 to 1×10^8 FCU per gram of wet mass) promotes the decrease of the pH [25]. Among the microorganisms used, *L. casei* was suitable for this process [26,27]. Therefore, ensiling could depend on sargassum properties such as salt, fermentable sugar, and nutrient content [23].

The predominant sargassum species in the Mexican Caribbean (*S. fluitans* and *S. natans*) contain high concentrations of sodium (17–38 g/kg), calcium (96–133 g/kg), and magnesium (10–18 g/kg), which are even higher than those reached in seawater (10.6, 0.40, and 0.38 g/kg for Na, Ca and Mg, respectively) [21]. The presence of these elements reflects a high salinity level, which could hinder the biological activity of *L. casei*. In contrast, the glucose content in sargassum dry mass is ~5 % [22], which is insufficient to reach an LA concentration typical of that of other silage materials (6 %– 10 % on a dry basis) [23]). The above shows that sargassum without pretreatment cannot be used as a substrate for lactic fermentation. Therefore, in the following sections, sargassum washing and additive incorporation (glucose and nutrients) are evaluated.

3.3 Sargassum washing

To remove the salt contained in sargassum, a washing process was developed. The salt content was indirectly measured by electrical conductivity (EC). In the first wash cycle, the EC reached 8.943 ± 0.176 mS/cm, which was reduced by ~85 % at the second wash (1.363 ± 0.022 mS/cm) (Fig. 1). From the third wash cycle, the EC presented similar values from 0.207 to 0.362 mS/cm. This indicates that two washing cycles are sufficient to remove up to 85 % of the initial salt concentration. Therefore, this pretreatment was implemented for sargassum ensiling.

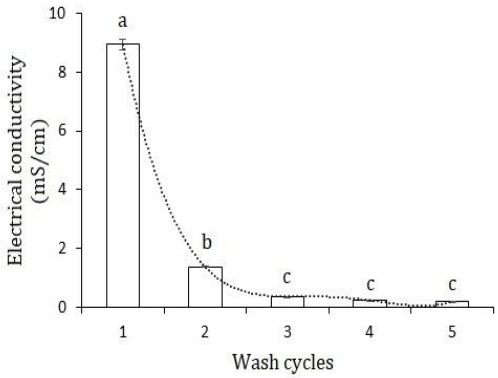

Figure 1. Electrical conductivity in washed sargassum. Different letters indicate significant differences (Tukey´s test, α = 0.5).

3.4 Effect of the carbon source addition on the silage of washed sargassum beds enriched with nutrients

In Section 3.2, it was determined that the contents of fermentable sugars and nutrients in sargassum are not sufficient for ensiling. For that, the effect of glucose addition on the silage of washed sargassum spiked with NS was evaluated. Sargassum silos with a glucose content of 1.6 %, 4 %, and 8 % on a wet basis (equivalent to 66, 149, and 261 mg per gram of dry mass (gdm), respectively) were evaluated. During ensiling (14 d), the material in the silos presented an EC between 3.5 and 8 mS/cm (Fig. 2A). After 3 days of ensiling, the pH decreased in all treatments (Fig. 2B) due to lactic acid production (~50 mg/gms) (Fig. 2C). However, after 7 days, the pH decreased, and LA production only continued in silos with at least 149 mg of glucose/gdm. At the end of the process, the lowest pH (~4.8) and the highest lactic acid production (~178 mg/gdm) were obtained by adding 261 mg of glucose/gdm. Increasing the glucose addition from 149 to 261 mg/gdm produced ~41 % more lactic acid, with a bioconversion rate of ~68 %.

The NS addition increased the sargassum EC; however, this did not affect the ensiling process. This suggests that non-washed sargassum could be ensiled by *L. casei* with the addition of an easily assimilated carbon source, such as glucose. The glucose content required to ensile sargassum

is higher than that required to ensile forages such as alfalfa (70–120 mg/gms) (Muck 1990). The above could be due to the sargassum buffer capacity, which limits the pH decrease [23]. Therefore, despite the high LA production in sargassum silos with a glucose content of 261 mg/gdm, the pH did not decrease to the value typically obtained in forage and crop silages (between 3.7 and 4.3) [23,29]. However, the conditions generated allow stability in the silo (Fig. 2B and 2C); therefore, the 8 % glucose addition (~261 mg/gms) is recommended for sargassum silage.

3.5 Silage sensitivity to washing and nutrient addition

To improve the process efficiency, its sensitivity to washing and NS addition was evaluated. For this, four treatments were implemented: 1) unwashed and water-impregnated (USW), 2) unwashed and NS-impregnated (USNS), 3) washed and water-impregnated (WSW), and 4) washed and NS-impregnated (WSNS) (Table 1). During ensiling, NS addition was essential for the pH decrease in the sargassum silo (Fig. 3A) since it increases the total production of AL (Fig. 3B) and AA (Fig. 3C). On the other hand, sargassum washing increases the AL production velocity without affecting the total production. Likewise, the washing of sargassum does not affect the production of AA (Fig. 3C) and ethanol (Fig. 3D).

After ensiling, the treatments USNS and WSNS reached an AL: AA ratio of 8.5 and 7.9, respectively. These values are higher than those obtained in other silage processes (from 2.5 to 3). The above could be due to the inoculant addition and the high initial glucose concentration, which promotes LA production, reaching AL: AA ratio values greater than 7 [23,30]. In this case, the heterolactic facultative *L. casei* [31] favors LA production when there are no substrate limitations [30]. In contrast, in the USNS and WSNS silos, AA and Et production remained within the ranges of 1 %–3 % and 0. 5%–1.5 % (dry basis), respectively. This indicates an appropriate ensiling process and a low or no propagation of microorganisms such as enterobacteria, clostridia bacteria [32], and/or yeasts [31]. The above results demonstrate that *L. casei* requires nutrient addition for proper sargassum ensiling, independent of sargassum washing.

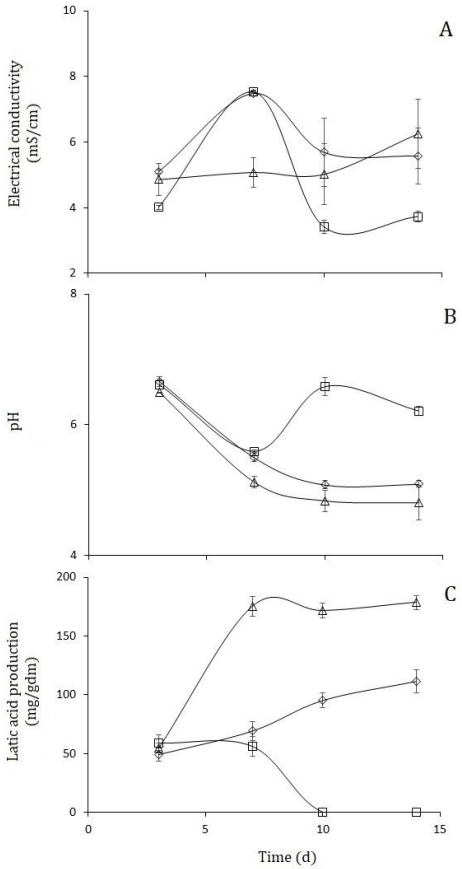

Figure 2. Kinetics of electrical conductivity (A), pH (B), and lactic acid production (C) in silos with 1.6 (squares), 4 (diamonds), and 8 % (triangles) glucose.

4. Conclusions

Sargassum conservation through ensiling may be the key to its industrial exploitation. The addition of an easily assimilable carbon source, such as glucose and a nutrient (NS) allows sargassum silage by *Lactobacillus casei*, decreasing the pH to ~4.8 and reaching a high bioconversion of glucose to lactic acid. Under these conditions, ensiling is

independent of the salt content in the sargassum, making desalination unnecessary.

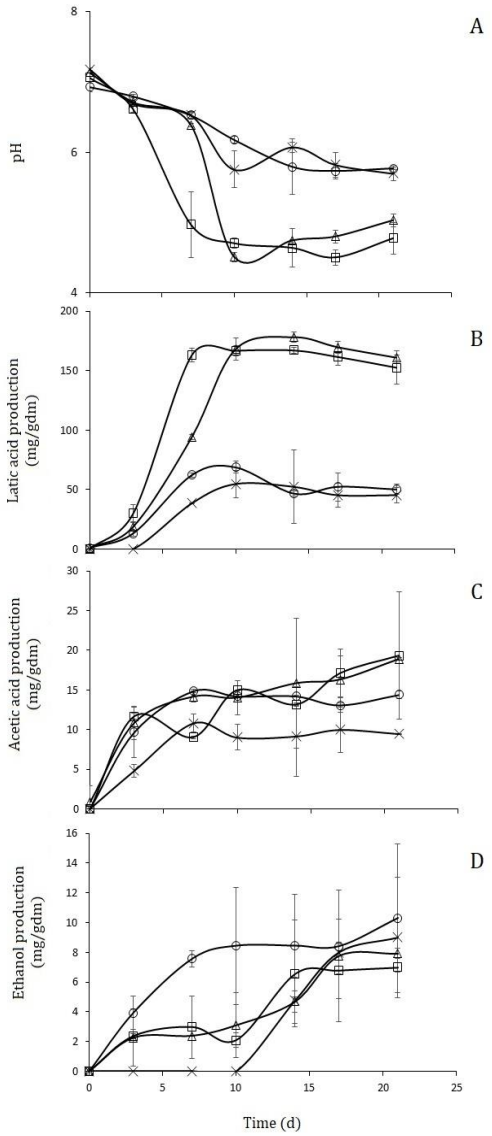

Figure 3. Kinetics of pH (A), lactic acid (B), acetic acid (C), and ethanol (D) production in silos packed with unwashed and water-impregnated (crosses), unwashed and NS-impregnated (triangles), washed and water-impregnated (circles), and washed and NS-impregnated (squares) sargassum.

5. Acknowledgments

Hernández-Zarco B. thanks the Mexican National Council for Science and Technology (CONACyT) for the scholarship.

6. References

[1] F. Méndez-González, A. Pichardo-Sánchez, B. Espinosa-Ramírez, N. Rodríguez-Durán, G. Bustos-Vázquez, L.V. Rodríguez-Durán, Valorization of nonnative aquatic weeds biomass through their conversion to biofuel, in: L. Ríos-González, A. Rodríguez-de-la-Garza, M. Medina-Morales, C. Aguilar (Eds.), Handbook of Research on Bioenergy and Biomaterials, 1st ed., Apple Academic Press, New York, 2021: pp. 271–282.

[2] N.F. Putman, R. He, Tracking the long-distance dispersal of marine organisms: sensitivity to ocean model resolution, J R Soc Interface. 10 (2013) 20120979. https://doi.org/10.1098/rsif.2012.0979

[3] M. Wang, C. Hu, B.B. Barnes, G. Mitchum, B. Lapointe, J.P. Montoya, The great Atlantic Sargassum belt, Science (1979). 365 (2019) 83–87. https://doi.org/10.1126/science.aaw7912

[4] B.I. van Tussenbroek, H.A. Hernández Arana, R.E. Rodríguez-Martínez, J. Espinoza-Avalos, H.M. Canizales-Flores, C.E. González-Godoy, M.G. Barba-Santos, A. Vega-Zepeda, L. Collado-Vides, Severe impacts of brown tides caused by Sargassum spp. on near-shore Caribbean seagrass communities, Mar Pollut Bull. 122 (2017) 272–281. https://doi.org/10.1016/j.marpolbul.2017.06.057

[5] V. Chávez, A. Uribe-Martínez, E. Cuevas, R.E. Rodríguez-Martínez, B.I. van Tussenbroek, V. Francisco, M. Estévez, L.B. Celis, L.V. Monroy-Velázquez, R. Leal-Bautista, L. Álvarez-Filip, M. García-Sánchez, L. Masia, R. Silva, Massive influx of pelagic Sargassum spp. on the coasts of the Mexican Caribbean 2014–2020: Challenges and opportunities, Water (Basel). 12 (2020) 2908. https://doi.org/10.3390/w12102908

[6] J.L. López Miranda, L.B. Celis, M. Estévez, V. Chávez, B.I. van Tussenbroek, A. Uribe-Martínez, E. Cuevas, I. Rosillo Pantoja, L. Masia, C. Cauich-Kantun, R. Silva, Commercial potential of pelagic Sargassum spp. in Mexico, Front Mar Sci. 8 (2021). https://doi.org/10.3389/fmars.2021.768470

[7] F. Méndez-González, J.J. Buenrostro-Figueroa, Sargazo, el potencial recurso del Mar Caribe, Mexican Journal of Technology and Engineering. 2 (2023) 14–17.

[8] K.K.A. Sanjeewa, N. Kang, G. Ahn, Y. Jee, Y.-T. Kim, Y.-J. Jeon, Bioactive potentials of sulfated polysaccharides isolated from brown seaweed Sargassum spp in related to human health applications: A review, Food Hydrocoll. 81 (2018) 200–208. https://doi.org/10.1016/j.foodhyd.2018.02.040

[9] G.A. Molina, M.A. González-Reyna, A.M. Loske, F. Fernández, D.A. Torres-Ortiz, M. Estevez, Weak shock wave-mediated fucoxanthin extraction from Sargassum spp. and its electrochemical quantification, Algal Res. 68 (2022) 102891. https://doi.org/10.1016/j.algal.2022.102891

[10] J.J. Milledge, P.J. Harvey, Ensilage and anaerobic digestion of Sargassum muticum, J Appl Phycol. 28 (2016) 3021–3030. https://doi.org/10.1007/s10811-016-0804-9

[11] K. Saravanan, S. Duraisamy, G. Ramasamy, A. Kumarasamy, S. Balakrishnan, Evaluation of the saccharification and fermentation process of two different seaweeds for an ecofriendly bioethanol production, Biocatal Agric Biotechnol. 14 (2018) 444–449. https://doi.org/10.1016/j.bcab.2018.03.017

[12] S. Surtharsan, S. Nishanthi, S. Srikrishnah, Effects of foliar application of seaweed (Sargassum crassifolium) liquid extract on the performance of Lycopersicon esculentum Mill. in Sandy Regosol of Batticaloa District Sri Lanka, Am Eurasian J Agric Environ Sci. 14 (2014) 1386–1396.

[13] A. Kumar, B. Vanlazarzova, S. Sridhar, M. Baluswami, Effect of liquid seaweed fertilizer of Sargassum wightii grev. on the growth and biochemical content of green gram (Vigna radiata (L.) R. wilczek), Recent Research in Science and Technology. 4 (2012) 40–45.

[14] C. Kalaivanan, V. Venkatesalu, Utilization of seaweed Sargassum myriocystum extracts as a stimulant of seedlings of Vigna mungo (L.) Hepper, Spanish Journal of Agricultural Research. 10 (2012) 466–472. https://doi.org/10.5424/sjar/2012102-507-10

[15] P. Vaghela, K. Trivedi, K.G.V. Anand, H. Brahmbhatt, J. Nayak, K. Khandhediya, K. Prasad, K. Moradiya, D. Kubavat, L.J. Konwar, V. Veeragurunathan, P.G. Grace, A. Ghosh, Scientific basis for the use of minimally processed homogenates of Kappaphycus alvarezii (red) and Sargassum wightii (brown) seaweeds as crop biostimulants, Algal Res. 70 (2023) 102969. https://doi.org/10.1016/j.algal.2023.102969

[16] S. Carrillo, A. Bahena, Casas M, Carranco M, Calvo C, E. Ávila, F. Pérez-Gil, El alga Sargassum spp. como alternativa para reducir el contenido de colesterol en el huevo, Revista Cubana de Ciencia Agrícola. 46 (2012) 181–186.

[17] H. Gojon-Báez, D. Siqueiros-Beltrones, H. Hernández-Contreras, In situ ruminal digestibility and degradability of Macrocystis pyrifera and Sargassum spp. In bovine livestock, Cienc Mar. 24 (1998) 463–481. https://doi.org/10.7773/cm.v24i4.762

[18] K.Y. Park, Y.H. Jo, J. Ghassemi Nejad, J.C. Lee, H.G. Lee, Evaluation of nutritional value of Ulva sp. and Sargassum horneri as potential eco-friendly ruminants feed, Algal Res. 65 (2022) 102706. https://doi.org/10.1016/j.algal.2022.102706

[19] J. Vijayakumar, R. Aravindan, T. Viruthagiric, Recent trends in the production, purification and applicatión of lactic acid, Chemical and Biochemical Engineering Quaterly. 22 (2008) 245–264.

[20] V. Hervé, J. Lambourdière, M. René-Trouillefou, D.A. Devault, P.J. Lopez, Sargassum differentially shapes the microbiota composition and diversity at coastal tide sites and inland storage sites on Caribbean Islands, Front Microbiol. 12 (2021). https://doi.org/10.3389/fmicb.2021.701155

[21] F. Fernández, C. Boluda, J. Olivera, L. Guillermo, E. Echeverria, A. Gómez, Análisis elemental prospectivo de la biomasa algal acumulada en las costas de la República Dominicana, Revista Centro Azúcar. 44 (2017) 11–22.

[22] A. Desrochers, S. Cox, H. Oxenford, B. van Tusenbroek, Sargassum uses guide: A resource for Caribbean researchers, entrpreneurs and policy makers, Centre for Resource Management and Environmental Studies, Barbados, 2020.

[23] L. Kung, R.D. Shaver, R.J. Grant, R.J. Schmidt, Silage review: Interpretation of chemical, microbial, and organoleptic components of silages, J Dairy Sci. 101 (2018) 4020–4033. https://doi.org/10.3168/jds.2017-13909

[24] R.E. Muck, Factors influencing silage quality and their implications for management, J Dairy Sci. 71 (1988) 2992–3002. https://doi.org/10.3168/jds.S0022-0302(88)79897-5

[25] G. Borreani, E. Tabacco, R.J. Schmidt, B.J. Holmes, R.E. Muck, Silage review: Factors affecting dry matter and quality losses in silages, J Dairy Sci. 101 (2018) 3952–3979. https://doi.org/10.3168/jds.2017-13837

[26] T.W. Kang, A.T. Adesogan, S.C. Kim, S.S. Lee, Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage, J Dairy Sci. 92 (2009) 732–738. https://doi.org/10.3168/jds.2007-0780

[27] K.G. Arriola, O.C.M. Queiroz, J.J. Romero, D. Casper, E. Muniz, J. Hamie, A.T. Adesogan, Effect of microbial inoculants on the quality and aerobic stability of bermudagrass round-bale haylage, J Dairy Sci. 98 (2015) 478–485. https://doi.org/10.3168/jds.2014-8411

[28] R. E. Muck, Dry matier level effects on alfalfa silage quality II. fermentation products and starch hydrolysis, Transactions of the ASAE. 33 (1990) 0373–0381. https://doi.org/10.13031/2013.31340

[29] T.F. Bernardes, L.G. Nussio, R.C. do Amaral, Top spoilage losses in maize silage sealed with plastic films with different permeabilities to oxygen, Grass and Forage Science. 67 (2012) 34–42. https://doi.org/10.1111/j.1365-2494.2011.00823.x

[30] L. Kung, M. Stokes, C. Lin, Silage additives, in: D. Buxton, R. Muck, J. Harrison (Eds.), Silage Science and Technology, American Society of Agronomy, Madison, 2003: pp. 305–360.

[31] R.E. Muck, E.M.G. Nadeau, T.A. McAllister, F.E. Contreras-Govea, M.C. Santos, L. Kung, Silage review: Recent advances and future uses of silage additives, J Dairy Sci. 101 (2018) 3980–4000. https://doi.org/10.3168/jds.2017-13839

[32] P. McDonald, A. Henderson, S. Heron, The Biochemistry of Silage, 2nd ed., Chalcombe Publications, Marlow, 1992. https://doi.org/10.1017/S0014479700023115