



BIODIESEL PRODUCTION WITH MACROPHYTES COLLECTED IN EUTROPHICATED SITES

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ABSTRACT

Objective: This research aimed to analyze the giant salvinia (*Salvinia molesta* D.Mitch) and water lettuce (*Pistia stratiotes* L.) potential, collected in the eutrophic environment for biodiesel production.

Theoretical framework: Macrophytes can cause environmental and economic damage in eutrophic environments due to excessive growth, being necessary for removal. In this sense, the destination of this biomass can be challenging.

Method: The moisture and lipid content of the macrophytes was evaluated and the yield of biodiesel produced via in situ transesterification by methyl and ethyl route for dry biomass was compared.

Results and conclusion: *S. molesta* showed the highest moisture content, with both macrophytes showing a significant difference ($p < 0.05$) between them and between the analyzed parts. *P. stratiotes* showed a lipid content significantly higher ($p < 0.05$). The yield of the biodiesel produced via in situ transesterification, by the ethylic route was higher ($p < 0.05$) for both biomasses, with *P. stratiotes* obtaining the best yield and *S. molesta* presenting the best use for the determined lipid content.

Implications of the research: *P. stratiotes* presented the best results for the production of biodiesel in the analyzed parameters, being a possible destination for this type of biomass.

Originality/value: The disposal of macrophytes from eutrophication means for biodiesel production is a new eco-friendly alternative for macrophyte infestations.

Keywords: Transesterification, Moisture Content, Lipids, Eutrophicated.

PRODUÇÃO DE BIODIESEL COM MACRÓFITAS COLETADAS EM LOCAIS EUTROFIZADOS

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RESUMO

Objetivo: Esta pesquisa teve como objetivo analisar o potencial da salvina gigante (*Salvinia molesta* D.Mitch) e da alface d'água (*Pistia stratiotes* L.), coletadas em ambiente eutrófico para produção de biodiesel.

Referencial teórico: As macrófitas podem causar danos ambientais e econômicos em ambientes eutróficos devido ao crescimento excessivo, sendo necessária sua remoção. Nesse sentido, a destinação dessa biomassa pode ser desafiadora.

Método: Avaliaram-se os teores de umidade e lipídios das macrófitas e comparou-se o rendimento do biodiesel produzido via transesterificação *in situ* pelas rotas metila e etila para biomassa seca.

Resultados e conclusão: *S. molesta* apresentou o maior teor de umidade, com ambas as macrófitas apresentando diferença significativa ($p < 0,05$) entre elas e entre as partes analisadas. *P. stratiotes* apresentou um conteúdo lipídico significativamente maior ($p < 0,05$). O rendimento do biodiesel produzido via transesterificação *in situ*, pela rota etílica foi maior ($p < 0,05$) para ambas as biomassas, com *P. stratiotes* obtendo o melhor rendimento e *S. molesta* apresentando o melhor aproveitamento para o teor lipídico determinado.

Implicações da pesquisa: *P. stratiotes* apresentou os melhores resultados para produção de biodiesel nos parâmetros analisados, sendo um possível destino para este tipo de biomassa.

Originalidade/valor: O descarte de macrófitas provenientes de meios de eutrofização para produção de biodiesel é uma nova alternativa ecologicamente correta para infestações de macrófitas.

Palavras-chave: Transesterificação, Umidade, Lipídios, Eutrofização.

PRODUCCIÓN DE BIODIÉSEL CON MACROFITAS RECOLECTADAS EN SITIOS EUTROFIZADOS

RESUMEN

Objetivo: Esta investigación tuvo como objetivo analizar el potencial de salvina gigante (*Salvinia molesta* D.Mitch) y lechuga de agua (*Pistia stratiotes* L.), recolectadas en el medio eutrófico para la producción de biodiesel.

Marco teórico: Las macrófitas pueden causar daños ambientales y económicos en entornos eutróficos debido al crecimiento excesivo, siendo necesarias para su eliminación. En este sentido, el destino de esta biomasa puede ser desafiante.

Método: Se evaluó la humedad y el contenido lipídico de las macrófitas y se comparó el rendimiento del biodiésel producido mediante transesterificación *in situ* por vía metílica y etílica para la biomasa seca.

Resultados y conclusión: *S. molesta* mostró el mayor contenido de humedad, con ambos macrófitos mostrando una diferencia significativa ($p < 0,05$) entre ellos y entre las partes analizadas. *P. stratiotes* mostró un contenido de lípidos significativamente mayor ($p < 0,05$). El rendimiento del biodiésel producido mediante transesterificación *in situ*, por la vía etílica, fue mayor ($p < 0,05$) para ambas biomassas, siendo *P. stratiotes* el que obtuvo el mejor rendimiento y *S. molesta* el que presentó el mejor uso para el contenido lipídico determinado.

Implicaciones de la investigación: *P. stratiotes* presentó los mejores resultados para la producción de biodiesel en los parámetros analizados, siendo un posible destino para este tipo de biomasa.

Originalidad/valor: La eliminación de macrófitos de los medios de eutrofización para la producción de biodiésel es una nueva alternativa ecológica para las infestaciones de macrófitos.

Palabras clave: Transesterificación, Contenido de Humedad, Lípidos, Eutrofizados.

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1 INTRODUCTION

Some species macrophyte species present invasive behavior that causes environmental imbalance. These species are sensitive to environmental changes, presenting changes in their development in eutrophic environments, increasing the growth rate, which increases decomposing organic matter in the water and reduces dissolved oxygen levels and light incidence (Coetzee et al. 2020; Santos et al., 2022).

Salvinia molesta D.Mitch is a macrophyte native of southwestern Brazil that invades natural and artificial water bodies, being considered one of the most harmful invasive species worldwide, causing economic and social damage (Li et al, 2018; Coetzee & Hill, 2020). The species *Pistia stratiotes* L. is also native to South America and is widely used for ornamental purposes, causing a great impact in tropical and subtropical regions when falling into riverbeds (Jaklič et al., 2020).

Destining these biomasses for biotechnological applications is extremely important to reduce harmful impacts on society and the environment, in this sense, the production of biofuels is an alternative due to the rapid growth of these macrophytes (Miranda et al, 2016; Gusain & Suthar, 2017; Anto et al, 2020). This waste disposal can be framed within the definition of sustainability (Zampier et al., 2022; Moura et al., 2023).

Olivera Junior et al. (2021) already analyzed the production of biodiesel with *S. auriculata* and Venu et al. (2019) with *Eichhornia crassipes* Mart Solms. While the works of Mubarak et al. (2016a, 2016b, 2021) explored the biodiesel production from *S. molesta* oil in India, to add this biodiesel to commercial diesel (Jain et al., 2022).

Biodiesel is one of these biofuels, which in addition to having socioeconomic implications by creating a destination for these biomasses, also helps to reduce the environmental impact associated with the use of fossil fuels (Mubarak et al., 2016a). This biofuel is formed by alkyl esters from the transesterification of long-chain carboxylic acids, from renewable vegetable or animal sources (Gondim et al, 2017). Its characteristics are similar to diesel, being safe to be stored, containing low sulfur content, being biodegradable (Cintra et al, 2017), and having lower pollutant emissions compared to fossil fuels (Barros et al, 2020).

Oilseeds are usually used for the production of biodiesel, such as corn, soy, peanuts, sunflower, and palm (Cardoso et al, 2020), being considered first-generation raw materials, with easy conversion, but with cultivation dispute with food production (Singh et al, 2020). According to the same authors, algae and macrophytes can be considered third-generation raw



materials, with the advantage of a high growth rate and the absence of competition with the food production chain.

However, one of the technical difficulties of allocating biomass from aquatic plants for this purpose is related to the lipid extraction stage (Anto et al, 2020). An alternative to avoid the lipid extraction step is direct transesterification, simplifying the production process and reducing the cost (Galina et al, 2020; Zorn et al, 2020).

Given what has been reported, this research aimed to verify the moisture content and yield of lipid extraction in *S. molesta* and *P. stratiotes* and to compare the yield of direct transesterification by methyl and ethyl routes of these dry biomasses to evaluate the potential of these macrophytes for the biodiesel production.

2 METHODOLOGY

2.1 SAMPLE COLLECTION

The collection of *S. molesta* was carried out in the lake of the Antenor Martins garden (S 22°13'47" W 54°49'58"), located in the urban area of Dourados-MS, Brazil, and later the samples were registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen) under registration number A77F799. The specimen was registered at UFGD under code 9668.

The collection of *P. stratiotes* took place in the lake of Anulpho Fioravante garden (S 22°14'3.1" W 54°47'47.52"), which is located in the urban region of Dourados-MS, Brazil, being registered in SisGen on registration number A57539D. The specimen was registered at UFGD under code 9669.

The plants were conditioned in buckets containing water from the collection place for transportation.

2.2 MOISTURE CONTENT

To determine the moisture content, the samples were cleaned in running water and dried at room temperature for 24 hours. Subsequently, the samples were crushed and placed in porcelain crucibles with previously determined weight and subjected to heating in an oven at 60 °C until constant mass. The moisture content of the submerged part of the plant, the aerial part, and the whole plant were determined.



The calculation of the moisture content was carried out according to Equation 1, where $U\%$ is the moisture content, M_1 is the weight of the porcelain crucible, M_2 is the weight of the porcelain crucible with wet biomass and M_3 is the weight of the crucible of porcelain with dry biomass.

$$U_{\%} = \left\{ \frac{[M_2 - (M_3 - M_1)]}{M_2} \right\} \times 100 \quad (1)$$

2.3 LIPID EXTRACTION

For the lipids extraction, the methodology described by Bligh & Dyer (1959), modified by D'Oca et al (2011) with adaptations was used. 0.1 g of dry biomass was weighed and 3 mL of a mixture of hexane: methanol (2:1 v/v) was added. The sample was centrifuged at a speed of 2000 rpm for 3 minutes and the supernatant was separated into a test tube with previously known mass and sent to an oven at 60 °C until constant weight. The quantification of lipids was performed according to Equation 2, where $L\%$ is the lipid content, M_1 is the mass of the test tube, M_2 is the tube with the extracted lipids and M_3 is the mass of the biomass used.

$$L_{\%} = \left(\frac{M_2 - M_1}{M_3} \right) \times 100 \quad (2)$$

To determine the best relationship between lipid content and moisture content, the ratio between these parameters was calculated, according to Equation 3.

$$Ratio = \left(\frac{L_{\%}}{U_{\%}} \right) \quad (3)$$

2.4 DIRECT TRANSESTERIFICATION

The methodology described by Galina et al (2020) was used in the production stage of methyl and ethyl esters. For each 5 g of dry biomass, 17 mL of methanol or ethanol, 20 mL of hexane, and 3 mL of concentrated sulfuric acid were added. The reaction took place in a water bath at 90 ± 1 °C for 60 minutes with magnetic stirring. After the end of the reaction, the supernatant was filtered with cotton, 35 mL of hexane was added to the biomass, and the sample was filtered again. Finally, phase separation was performed with the addition of 50 mL of



distilled water. It was decanted for 30 minutes and the supernatant was separated into a beaker of previously known mass, keeping it in an oven at 60°C until constant mass.

The yield calculation was performed according to Equation 4, using R% as the reaction yield, M1 as the mass of the beaker, M2 as the mass of the glassware with biodiesel, and M3 as the mass of the sample used in the reaction.

$$R_{\%} = \left(\frac{M_2 - M_1}{M_3} \right) \times 100 \quad (3)$$

To determine the efficiency of direct transesterification, Equation 4 was used, where E% is the efficiency of direct transesterification about the lipid content.

$$E_{\%} = \frac{R_{\%} \times 100}{L_{\%}} \quad (4)$$

2.5 STATISTICAL ANALYSIS

Statistical analysis was performed using the R platform (R Core Team, 2021). The normality of the replicas was analyzed using the Ryan-Joiner test and a study of variances where it was found that the data are parametric. The analysis by ANOVA was performed to verify if there was a significant difference between the treatments of the analyzes employed and finally the Tukey test was used with a significance of 0.05 for the samples with a significant difference.

3 RESULTS AND DISCUSSION

The environment of both collections showed signs of eutrophication. The eutrophic environment has an excess of nutrients that result in the proliferation of macrophytes, however, the high rate of decomposition of organic matter consumes the oxygen dissolved in the water, directly affecting the aquatic fauna (Oliveira Junior et al., 2021). The presence of a dead fish floating among the macrophytes in the Arnulpho Fioravanti garden was observed, probably as a result of eutrophication (Figure 1).

**Figure 1**

Dead fish indicating eutrophication at the P. stratiotes collection place



Source: Prepared by the authors (2023)

The moisture content of macrophytes is a relevant factor when considering the use of biomass. The different parts of the plant showed significantly different moisture contents ($p < 0.05$) among themselves, also between *S. molesta* and *P. stratiotes* for all parts (Table 1).

Table 1

The moisture content of macrophytes

Part of the plant	Moisture content (%) $\pm$ SD
SPSM	96.51 $\pm$ 0.26a
APSM	94.65 $\pm$ 0.15b
WPSM	93.57 $\pm$ 0.28c
SPPS	90.23 $\pm$ 0.12e
APPS	89.57 $\pm$ 0.09f
WPPS	90.74 $\pm$ 0.16d

SPSM = Submerged part of *S. molesta*; APSM = Aerial part of *S. molesta*; WPSM = Whole part of *S. molesta*; SPPS = Submerged part of *P. stratiotes*; APPS = Aerial part of *P. stratiotes*; WPPS = Whole part of *P. stratiotes*; SD = Standard Deviation. Means followed by different letters in the column differ from each other by the Tukey test with a level ($p < 0.05$) of significance.

Source: Prepared by the authors (2023)

The submerged and aerial parts of the macrophytes showed different results, the rhizomes have a higher moisture content. It is plausible that this difference is a consequence of the position and function performed by each structure on the water surface. As they are free-floating emersed aquatic plants, the rhizomes, and stolons are submerged in the water at all times, while the aerial part is above the surface (Demarchi et al., 2018). Although the leaves,



which correspond to the aerial part, also capture nutrients from the water body, the roots are the main means of absorbing nutrients and water (Granéli & Solander, 1988; Nichols & Keeney, 1976).

P. stratiotes is a macrophyte belonging to the angiosperm phylum, having a more uniform vascularization throughout its body compared to *S. molesta*, which belongs to the pteridophyte phylum (Nabors, 2012). It was possible to observe a difference in humidity between the parts of both macrophytes. *P. stratiotes* had the lowest moisture content for all parts (Table 1). When comparing with the data obtained in the literature for *S. molesta* (Table 2), it is possible to verify that the moisture content obtained is close to that reported.

Table 2

The moisture content of macrophytes

Species	Moisture Content (%)	Observation	Reference
<i>P. stratiotes</i>	6.70%	Sample previously dry	Adelakun et al. (2016)
<i>S. molesta</i>	92.97%	No observations	O'Sullivan et al. (2010)
<i>Azolla filiculoides</i>	87.70%	The method with sun exposure for 4 days	Miranda et al. (2016)
<i>Cabomba caroliniana</i>	93.79%	No observations	O'Sullivan et al. (2010)
<i>Eichhornia crassipes</i>	85.30%	No observations	Brunerová; et al. (2017)
<i>Hydrilla verticillata</i>	88.50%	No observations	Brunerová; et al. (2017)
<i>Myriophyllum spicatum</i>	89.60%	No observations	Brunerová; et al. (2017)

Source: Prepared by the authors (2023)

The high moisture content of the species studied leads to the need for water depletion, since the percentage that can be extracted from lipids is reduced, making it difficult the obtaining lipids. However, this process must be carried out correctly, as inadequate drying processes can lead to contamination by fungi and bacteria, in addition, it can lead to the destruction and alteration of the active principles, thus making the plant material inappropriate for use, as mentioned by Amaral et al. (2003).

The high presence of water can also result in the hydrolysis of triglycerides into free fatty acids, culminating in saponification, especially when basic catalysis is used (Sitepu et al, 2020).



Thus, the high moisture content of the macrophytes is one of the main challenges for making biodiesel production viable. It is reported in the review by Mallick et al. (2016) that microalgae have moisture contents greater than 80%, thus, the challenges for the development of biodiesel with these biomasses are similar in this parameter. The study carried out by Mubarak et al. (2016a) showed higher lipid levels for *S. molesta*, possibly due to the conditions of the collection place.

For the other experiments, the use of the whole plant was determined due to its lower moisture content, resulting in better use of the biomass. The best extraction yield and the best relation between lipid content and moisture content were obtained for *P. stratiotes*, with a significant difference ($p < 0.05$) (Table 2).

Based on the moisture content (Table 1) and lipid content (Table 3) it was found that the lipids extraction for subsequent transesterification to obtain biodiesel is laborious, however direct transesterification can avoid the lipid extraction step.

Table 3

Lipid content of the macrophytes

Species	Lipid content (%) $\pm$ SD	RLM
<i>S. molesta</i>	4.43 $\pm$ 0.42b	0.047
<i>P. stratiotes</i>	5.90 $\pm$ 0.16a	0.065

RLM = Relation between lipid content and moisture; SD = Standard Deviation. Means followed by different letters in the column differ from each other by the Tukey test with a level ($p < 0.05$) of significance. Source: Prepared by the authors (2023)

The lipid levels obtained are higher than that reported by Oliveira Junior et al. (2021) for the *Salvinia auriculata* Aub collected, however, the authors obtained higher levels when cultivating with biosolids, vinasse, and N:P:K, indicating the possibility of increasing the lipid content of *S. molesta* and *P. stratiotes* through controlled cultivation.

Regarding *in situ* transesterification, the ethyl route was significantly higher ($p < 0.05$) compared to the methyl route for both species, with *P. stratiotes* being 0.97% higher to *S. molesta* in the ethyl route ($p < 0.05$) and there was no significant difference between the two species for the methyl pathway ($p > 0.05$) (Table 4).

**Table 4***The yield of in situ transesterification with different alcohols*

Part of the plant	Yield (%) $\pm$ SD	Efficiency (%)
BMSM	2.38 ± 0.10^c	53.72
BESM	3.07 ± 0.11^b	69.30
BMPS	2.00 ± 0.25^c	33.90
BEPS	4.04 ± 0.05^a	68.47

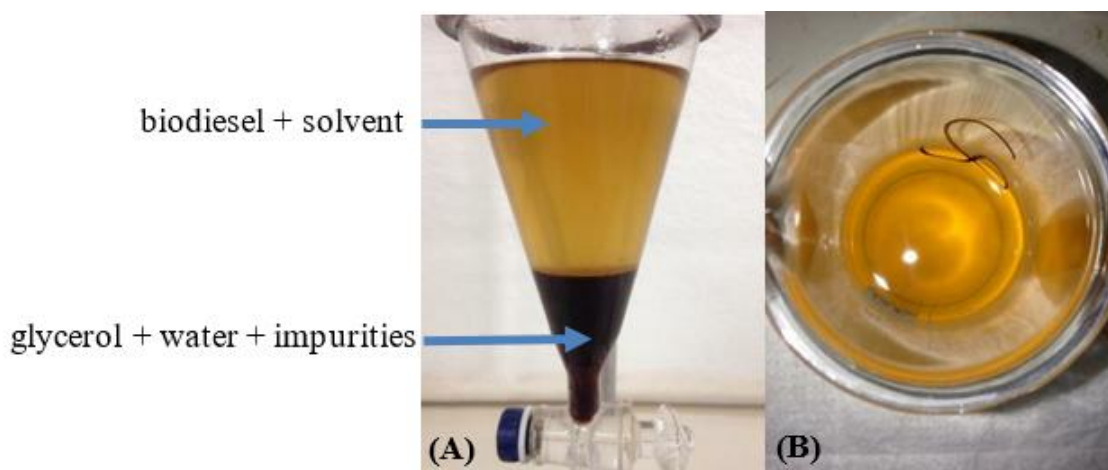
BMSM = Biodiesel produced by methylic route of *S. molesta*; BESM = Biodiesel produced by ethylic route of *S. molesta*; BEPS = Biodiesel produced by methylic route of *P. stratiotes*; BEPS = Biodiesel produced by ethylic route of *P. stratiotes*; SD = Standard Derivation.

Source: Prepared by the authors (2023)

It was noted that the phase rich in biodiesel (Figure 2), produced from the macrophytes studied, and from the different alcohols, did not present impurities such as sediments, chlorophyll pigments, or color change.

Figure 2

The aspect of the produced biodiesel. (A) The two-phase system formed in the phase separation; (B) phase enriched with biodiesel



Source: Prepared by the authors (2023)

The yield of *P. stratiotes* was higher for direct transesterification (Table 4), as well as for lipid extraction (Table 3). When analyzing the efficiency of direct transesterification, it is possible to observe that *S. molesta* and *P. stratiotes* by ethyl route showed the best results, whereas *P. stratiotes* with methanol showed the worst relationship between the yields of lipid contents and direct transesterification (Table 4).



Ethanol is one of the most relevant biotechnological products in the Brazilian scenario today, being a sustainable and low-cost raw material (Santos et al., 2019), the use of ethanol for direct transesterification produces a lower environmental impact, less cost and generates a higher yield for the dry biomass of *S. molesta* and *P. stratiotes* (Table 4).

Direct transesterification via the ethylic route is promising for both samples considering the yield obtained (Table 4) about the lipid content (Table 3).

4 FINAL CONSIDERATIONS

S. molesta and *P. stratiotes* are fast-growing macrophytes that can be harmful to the society and environment, however, biodiesel production is an alternative for the destination of this biomass. This study highlights the possible use of macrophytes as a sustainable alternative for the disposal of macrophytes from eutrophic regions. This can help in the management of excessive plant growth in eutrophic environments, reducing the social and environmental impact.

From this research, it is possible to verify that it is possible to obtain biodiesel from the *S. molesta* and *P. stratiotes* macrophytes, with the ethylic route being the most efficient for these biomasses. However, the high moisture content of macrophytes is a challenge to make the product viable at an industrial level, suggesting future studies that can explore alternatives to avoid this problem. Another relevant line of research is the viability of this product is the cultivation effect on the lipid content of macrophytes.

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