ARTICLE

IDENTIFICATION AND EVALUATION OF THE BIOTECHNOLOGICAL POTENTIAL OF *DESMODESMUS ARMATUS* (CHODAT) E.H. HEGEWALD ISOLATED FROM THE ECUADORIAN AMAZON

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Abstract: Currently, there is a strong trend toward developing biofuels from microalgae. This study identified and evaluated the biotechnological potential of *Desmodesmus armatus* (Chodat) E.H. Hegewald, isolated from the Ecuadorian Amazon, for bioremediation and biofuel production. Molecular and morphological identification of the L60cmC1B-17519 strain, obtained from the Limoncocha Lagoon in the Sucumbios province of Ecuador, was conducted using bibliographic comparisons, ITS2-PCR, and maximum likelihood phylogenetic analysis. This approach indicates that the strain isolated belongs to the *D. armatus* species. A ten-day culture was conducted in 1L batch photobioreactors with BG-11 medium, yielding 2.36 g.L-1 of biomass with a productivity of 0.24 g.L-1.d-1. Nutrient removal, measured in terms of nitrates, phosphates, and chemical oxygen demand, was monitored daily, with removal efficiencies of 55.35%, 68.31%, and 81.13%, respectively. Lipid content was evaluated from wet and dry biomass using chloroform and ethyl acetate as organic solvents. The best results were obtained with the chloroform (1:2) mixture, yielding lipid percentages of 21.50% from dry biomass. Additionally, free fatty acids were analyzed, with the highest yield of 52.80%, using chloroform extraction. These findings suggest that this microalga holds potential for biotechnological applications and highlight the need for future research.

Key-words: *Desmodesmus armatus*, bioremediation, renewable fuels.

IDENTIFICAÇÃO E AVALIAÇÃO DO POTENCIAL BIOTECNOLÓGICO DE *DESMOSESMUS ARMATUS* (CHODAT) E.H. HEGEWALD ISOLADO DA AMAZÔNIA EQUATORIANA

Resumo: Atualmente, há uma tendência crescente para o desenvolvimento de biocombustíveis a partir de microalgas. Este estudo avaliou o potencial biotecnológico de *Desmodesmus armatus* (Chodat) E.H. Hegewald, isolado da Amazônia equatoriana, para biorremediação e produção de

biocombustíveis. A identificação molecular e morfológica da cepa L60cmC1B-17519, obtida da Lagoa Limoncocha, na província de Sucumbíos, no Equador, foi realizada usando ITS2-PCR e análise filogenética, confirmando que a cepa pertence à espécie *D. armatus*. O cultivo de dez dias em fotobiorreatores de batelada de 1L com meio BG-11 produziu 2,36 g.L-1 de biomassa com produtividade de 0,24 g.L-1.d-1. A remoção de nutrientes, como nitratos, fosfatos e demanda química de oxigênio, teve eficiências de 55,35%, 68,31% e 81,13%, respectivamente. O teor de lipídios foi extraído de biomassa úmida e seca usando clorofórmio e acetato de etila. Os melhores resultados foram obtidos com a mistura de clorofórmio (1:2), com 21,50% de lipídios da biomassa seca. A extração com clorofórmio também obteve o maior rendimento de ácidos graxos livres, com 52,80%. Esses resultados indicam que essa microalga tem potencial para aplicações biotecnológicas e reforçam a importância de pesquisas futuras.

Palavras-chave: *Desmodesmus armatus*, biorremediação, combustíveis renováveis.

Introduction

The increase in the population of the world and the rise in living standards are the main reasons for the surge in global energy consumption and demand, which is primarily met by fossil fuels (Acar & Dincer, 2020). However, using these fuels harms the environment due to the release of greenhouse gases, such as carbon dioxide, methane, chlorofluorocarbons, nitrous oxide, and ozone, into the atmosphere (Al-Ghussain, 2019). This situation has led to significant interest in developing sustainable alternatives and new technologies to meet energy demand, such as biofuels like biodiesel (Khan et al., 2018).

There are three generations of raw materials used for producing biodiesel. The first generation is derived from sources like soybean and palm oil (Minhas et al., 2020; Melo Lisboa et al., 2020). The second generation comes from salmon oil, tobacco seeds, and straw materials. Finally, the third generation is produced from the biomass of microorganisms, including microalgae, yeasts, and fungi. Microalgae are autotrophic-heterotrophic photosynthetic microorganisms capable of converting solar energy and synthesizing carbon compounds by fixing CO2 (Maity et al., 2014). They are found in various bodies of water, such as lakes, ponds, and seas, and can also thrive in terrestrial environments due to their high adaptability (Abdel-Raouf, 2012; Sharma & Sharma, 2017; Maltsev et al., 2021). Microalgal biomass produces three main biochemical components: carbohydrates, proteins, and lipids. It is known that microalgae synthesize and accumulate lipids more rapidly and in greater amounts than terrestrial plants, owing to their high growth rates. These lipid concentrations vary depending on the species evaluated (Abdel-Raouf, 2012; Jimenez-Lopez et al., 2021).

Microalgae are studied as a major source of lipids, useful for producing biodiesel, as their lipid synthesis can be manipulated by modifying the culture medium (Brindhadevi et al., 2021). Microalgal lipids contain twice the energy per carbon atom compared to carbohydrates, which directly translates to a twofold increase in the energy content of the fuel (Brennan & Owende, 2010). Certain characteristics must be considered when selecting a microalga for biodiesel production, including high biomass production, lipid yield, and self-sedimentation efficiency (Rosenberg al., 2008; Jimenez-Lopez et al., 2021). The lipid content varies depending on the microalgal species, with some reaching up to 75% of their dry weight (Mata et al., 2010; Park et al., 2011). It is recommended to use microalgae with a lipid content between 20% and 50%, along with good biomass productivity (Khan et al., 2018).

The most commonly used microalgal genera in biofuel production are *Chlorella Beijerinck; Scenedesmus Meyen; Ankistrodesmus Corda; Euglena Ehrenberg; Chlamydomonas Ehrenberg; Oscillatoria Vaucher ex Gomont; Micractinium Fresenius; Golenkinia Chodat; Phormidium Kützing ex Gomont; Botryococcus Kützing; Spirulina Turpin ex Gomont; Nitzschia Hassall; Navicula Bory; and Stigeoclonium Kützing(*Minhas et al., 2020). However, other genera, such as *Desmodesmus* (Chodat) S.S.An, T.Friedl & E.Hegewald, also show high biomass production and lipid yield. This microalga is prevalent in eutrophic lakes and is commonly used to indicate the degree of contamination in these systems (Huang et al., 2012).

Cultivating microalgae for biomass generation to produce biodiesel can be expensive due to the large volumes of water required for industrial-scale production and the associated equipment and procedures. Therefore, using wastewater as a nutrient source is a viable alternative, given its potential in biodiesel production and its ability to reduce the overall cost of the process (Medrano-Barboza et al., 2021), particularly in Amazonian regions where ecological and anthropic conditions favor the presence of microalgae (Navas-Flores et al., 2021). Another way to reduce costs is by using wet bi-

omass during extraction, requiring no more than 10% water content after dehydration for this type of process. However, in typical centrifugation and dehydration processes, the microalgae pellet retains at least 60% water (Pan et al., 2017; Suparmaniam et al., 2019).

These challenges in biodiesel production highlight the importance of exploring new methods and species of microalgae, especially if the microorganism is autochthonous, opening the possibility of studying their natural potential for biotechnology application (Castelo, 2018; Khan et al., 2018). Therefore, this research aimed to study a strain of the microalga *Desmodesmus armatus* (Chodat) E.H. Hegewald, isolated from the lagoon of the Limoncocha Biological Reserve, located in the Ecuadorian Amazon, and identified by molecular and morphological tools, to evaluate its biomass and lipid-generating potential as raw material for biodiesel production and bioremediation applications.

Material and Methods

Isolation and culture

Water samples were collected from the Limoncocha lagoon within the Limoncocha Biological Reserve, located in the northeastern Ecuadorian Amazon, Sucumbíos Province (Fig. 1). The reserve primarily protects the Limoncocha lagoon, together with surrounding wetlands, swamp areas, and humid tropical forests. The lagoon shores and swamp zones host flora and fauna adapted to the permanent water-forest interaction. In the surrounding area, local livelihoods include subsistence agriculture such as cacao, banana, and maize, and the lagoon is also a major attraction for ecotourism. Samples were collected from the center of the lagoon using a Van Dorn (vertical water bottle). The water samples were stored and transported at 4°C, keeping the cold chain.

Prior to cultivation, each water sample was centrifuged (BOECO C-28A centrifuge) at 3500 × g for 4 min to concentrate microorganisms. Aliquots of 100 µL were then spread-plated under sterile conditions using a Drigalski spatula onto solid selective media, BG11 and BBM, 1.4% agar (Andersen, 2005). Plates were incubated at 15 °C - 19 °C (room temperature for Quito, Ecuador) under a 12:12 h light:dark photoperiod at 60 µmol.m-².s-1. Individual colonies were successively subcultured until morphologically confirmed axenic cultures were obtained and checked by light microscopy (Leica DM750) with structures mounted in Aquatex®. For institutional storage, the code L60cmC1B-

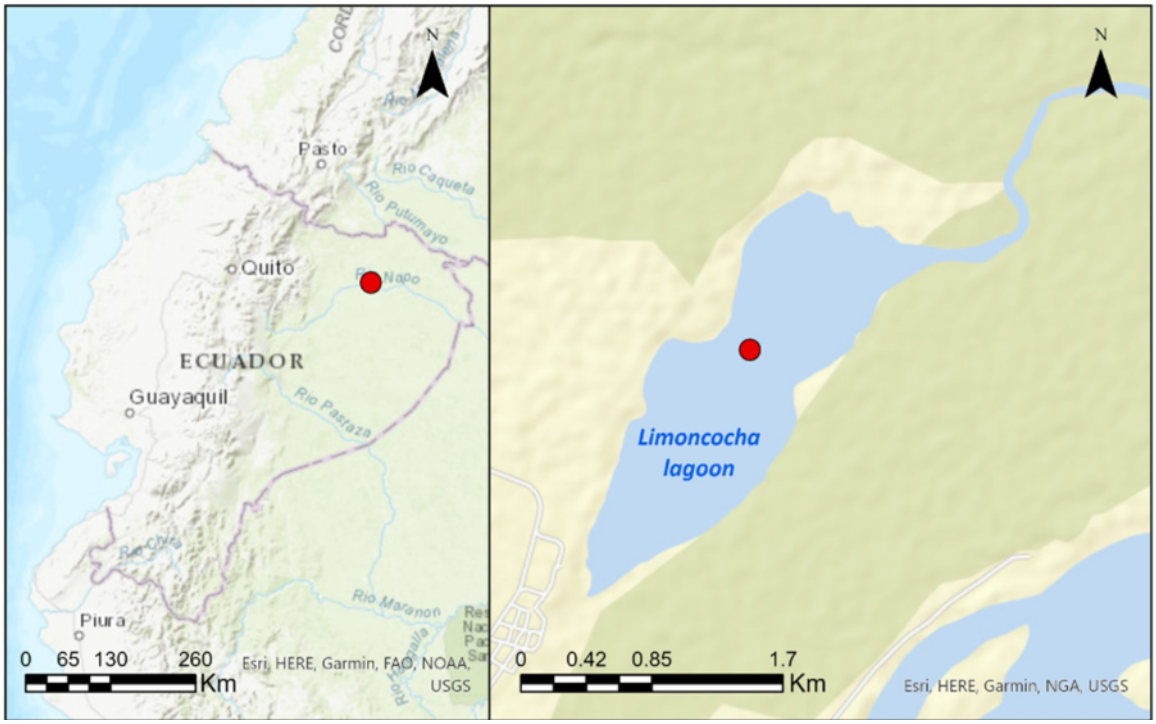

Fig. 1. Location of the sampling site in Ecuador. The left panel shows the geographic position of the study area within the country, and the right panel shows the sampling point (red circle) at the center of the lagoon, within the Limoncocha Biological Reserve (Sucumbíos Province, northeastern Ecuadorian Amazon).

17519 was assigned.

Morphological identification

The morphological identification was made using a EUROMEX PC/DIC binocular microscope equipped with a CMEX-PRO 18 camera at 40x, according to the techniques of Clarke & Eberhardt (2002). The taxonomic identification was made by analyzing the morphological variability of 10 specimens isolated from the culture to determine the shape of the cenobium with the number of cells, the length, width, shape, and the location of the chloroplasts. The presence and length of the spines were also considered. The genus and species were identified by comparison with the original descriptions proposed by (Geraldo et al., 2015) and (Hentschke & Torgan, 2010), in addition to the comparison with records available in the algae database (https://www.algaebase.org) (Guiry & Guiry, 2025).

Molecular identification and phylogenetic analysis

Amplifications of the 18S and Internal Transcribed Spacer ITS2 regions and contigs generated for the phylogenetic analysis were performed as described by Encalada-Rosales et al. (2022). Briefly, a colony polymerase chain reaction (PCR) was conducted with the ITS3-4 and the CHLORO primers (White et al., 1990; Moro et al., 2009). The amplicons were purified and sequenced by the Sanger Dideoxy-nucleotide method (MacroGEN®). The contigs obtained were assembled into a consensus sequence using the GeneStudio Professional Edition program.

A Basic Local Alignment Search Tool (BLAST) of our sequence (Accession code OR041503 stored at https://www.ncbi.nlm.nih.gov/genbank/) was performed to find the sequences with high molecular identity from the National Center for Biotechnology Information (NCBI) databases. The sequence alignments,

Tab 1. Medium BG-11 composition

Component	Stock Solution ($g.L^{-1}$ dH_2O)	Quantity Used	Concentration in Final Medium
Fe Citrate solution		1 mL	
Citric acid	6	1 mL	3.12E-3
Ferric ammonium citrate	6	1 mL	3E-5
$NaNO_3$	-	1.500 g	1.76E2
$K_2HPO_4 \cdot 3H_2O$	40	1 mL	1.75E-4
$MgSO_4 \cdot 7H_2O$	75	1 mL	3.04E-4
$CaCl_2 \cdot 2H_2O$	36	1 mL	2.45E-4
$NaCO_3$	20	1 mL	1.89E-4
$MgNa_2EDTA \cdot H_2O$	1.0	1 mL	2.79E-6
Trace metals solution		1 mL	
H_3BO_3	-	2.860 g	4.63E-5
$MnCl_2 \cdot 4H_2O$	-	1.810 g	9.15E-6
$ZnSO_4 \cdot 7H_2O$	-	0.220 g	7.65E-7
$CuSO_4 \cdot 5H_2O$	79.0	1 mL	3.16E-7
$Na_2MoO_4 \cdot 2H_2O$	-	0.391 g	1.61E-6
$Co(NO_3)_2 \cdot 6H_2O$	49.4	1 mL	1.70E-7

phylogenetic, and molecular evolutionary analyses were conducted using MEGA version X (Kuma et al., 2018; Stecher et al., 2020). Two sequences were included as outgroups in the ITS analyses (*Ankistrodesmus sp.* and *Ankistrodesmus falcatus* (Corda) Ralfs), while the ingroup microalgae sequences were constituted by *Desmodesmus, Scenedesmus, and Pediastrum Meyen.* The matrix alignment sequences with the DNA markers were analyzed to construct a phylogenetic tree under the maximum likelihood analysis method and the nucleotide substitution model (K2 + G), to find a molecular identification under the evolutionary relationships of the organisms. The accession codes of the ingroup microalgae species are shown on each taxon label on the tree.

Algal growth determination and biomass production

Liquid cultures were conducted in triplicate using photobioreactors containing 1 L of BG-11 medium (Andersen, 2005) (Tab. 1) for ten days, using an initial inoculum of 1.1·E6 cells.mL-1. The bioreactors were kept at a temperature between 15 °C and 19 °C. A 12:12 light cycle and a light intensity of 60 µmol.m-2.s-1 were provided, with a constant aeration supply of 4.2 L/min using a JAD S-4000B. In addition, the culture was kept at a neutral pH. To determine the cell growth curve, a daily count was performed using a Neubauer chamber in a binocular microscope. The cell concentration was calculated by applying equation (1).

Dry weight was determined using the method of Arredondo-Vega & Voltolina (2007). 1 mL of culture was taken and placed on a filter paper that was previously weighed. The sample was left to dry at 60 °C in a Memmert SM 200 stove until constant weight. The difference in total weight was recorded. At the end of the culture, all the obtained biomass was harvested and washed twice using deionized water to remove all the salt residues. The biomass was concentrated by centrifugation for 30 min at 4500 g in a BOECO C-28A centrifuge. Half of the biomass was kept wet, and the other half was dried in the stove at 60 °C until constant weight for further analysis.

The parameters volumetric productivity (VP) and specific productivity (SP)(d-1) of biomass were calculated with equation (2) and equation (3), respectively. The specific growth rate (µ) was calculated as the slope of the straight line in the exponential growth phase when plotting the natural logarithm of the biomass concentration versus culture time according to equation (4). Where NT is the number of cells at the end of the log phase, N0 is the number of cells at the start of log phase, TT is the final day of log phase, and T0 is the starting day of log phase (Akgül, 2020). Duplication time was calculated according to the equation (5)

Determination of the nutrient removal

Daily culture samples of 15 mL were centrifuged at 4500g in a BOECO C-28A centrifuge for 5 minutes. The supernatant was collected to determine the chemical parameters. Standardized protocols of the HACH Commercial House were used to determine phosphates (reactive phosphorus) (PO_4^{-3}) (HACH 8114), chemical oxygen demand (COD) (HACH 8000), and nitrates (NO_3^-) (HACH 8039) (HACH, 2000) using a HACH DRB 200 digestor and a HACH DR5000 spectrophotometer. To determine the nutrient removal efficiency, equation (6) was used. The experiments were performed in triplicate.

Determination of the lipid percentage

The percentage of lipids was determined following the Bligh and Dyer method (Smedes & Thomasen, 1996). 6 mL of high-performance liquid chromatography grade (HPLC grade) organic solvents, such as the mixture chloroform:methanol (1:2) and ethyl acetate, were used with 0.25 g of dry and wet biomass. A first sonication was applied to the biomass using a SONICS VibracellTM sonicator and solvent for a single cycle of 45 minutes (59 s, 30 s), left to stand for 24 hours at 4 °C, protected from light. Subsequently, a second sonication was carried out, and the extract was recovered and washed with 0.58% w/w of NaCl solution. The extract was centrifuged for 30 min at 4000 g, and finally, the organic phase was placed in a

$$Concentration = \frac{Totalcellscounted}{Totalofsquares} \times 1000 \times dilution \quad (1)$$

$$VPBiomass = \frac{Drybiomassweight[g]}{Culturevolume[L] \times Culturetime[d]} \left[\frac{g}{L \cdot d}\right] \quad (2)$$

$$SPBiomass = \frac{VPBiomass\left[\frac{g}{L \cdot d}\right]}{Biomassconcentration\left[\frac{g}{L}\right]} [d^{-1}] \quad (3)$$

$$\mu = \frac{Ln\frac{N_T}{N_0}}{T_T - T_0} [d^{-1}] \quad (4)$$

$$t_D = \frac{Ln[2]}{\mu} [d] \quad (5)$$

$$Nutrientremovalefficiency(\%) = \frac{IC - FC}{IC} \times 100 \quad (6)$$

previously weighed tube to dry at 68 °C in a stove until constant weight.

The final lipid percentage (wt%) was the average of the triplicates and the calculation applying equation (7), where T2 is the weight of a sample tube with the dry lipids (g), T1 is the weight of the empty and weighed sample tube (g), and W is the initial weight of the sample (g). The volumetric productivity (VP) of lipids was calculated with equation (8). For the specific productivity (SP) (d-1), the VP was divided by the biomass concentration (equation 9). All the experiments were carried out in triplicate.

$$wt\%lipidsandFFA = \left[\frac{T2-T1}{W}\right] \times 100 \quad (7)$$

$$VPLipids = \frac{Dryweight[g]}{culturevolume[L]xculturetime[d]}\left[\frac{g}{L \cdot d}\right] \quad (8)$$

$$SPLipids = \frac{VPLipids\left[\frac{g}{Ld}\right]}{biomassconcentration\left[\frac{g}{L}\right]}\left[d^{-1}\right] \quad (9)$$

Determination of the free fatty acids (FFA)

The FFA were determined according to (Tanzi et al., 2013) in triplicate, using the mixture chloroform:methanol (1:2) and ethyl acetate HPLC grade; with 0.50 g of dry biomass and 0.10 g of KOH. It was refluxed at 60 °C for 5 hours at 300 rpm. 5 mL of solvent used in the previous step was filtered and 5 mL of distilled water was added. The solution was transferred to a separatory funnel; the gases were released and allowed to stand for ten minutes. The organic phase was extracted in a previously weighed tube and then dried at 68 °C until constant weight.

The final percentage was obtained by making an average of the triplicates and applying equation (6), T2 is the weight of a sample tube containing the dry FFA (g), T1 is the weight of the empty sample tube (g), and W is the initial weight of the sample (g).

Statistical analysis

The cell concentration, optical density, biomass, and nutrient consumption were expressed as mean ± standard deviation (X ± SD). The means of the different experimental groups were compared using the nonparametric rank-based Kruskal-Wallis test in Statgraphics Centurion 18 (v18.1.16; Statgraphics Technologies, Inc., The Plains, VA, USA) to determine whether there were statistically significant differences between two or more groups of an independent variable in an ordinal or continuous dependent variable. Differences were considered statistically significant if the mean differences between each group presented $p < 0.05$ values.

Results

Isolation and morphological identification

The presence of a single microalgae strain was confirmed by microscopic observations. Cultures were found axenic, as no bacterial colonies were observed in the Petri dishes and under the microscope. Axenic colonies of the isolated microalga (L60cmC1B-17519) were obtained on BG-11 agar in Petri dishes (Fig. 2A). Cells typically formed coenobia or groups of two to four cells (Fig. 2B). A four-celled coenobium and a representative individual cell with its morphometric measurements at 100× magnification are shown in Fig. 2C.

The outer cells are composed of three short, slightly curved spines. The inner cells have short, linearly arranged spines, a parietal chloroplast, and a pyrenoid. The representative cell in Fig. 2C measured 5.7 µm in length, 4.6 µm in width, and 4.2 µm in spine length. These dimensions and morphological traits were consistently recorded across 45 evaluated coenobia. The analyzed strains exhibited a range of measurements consistent with those reported in the literature for the *Desmodesmus genus*. The length-to-width ratio and spine measurements were compared with the descriptions provided by (Hentschke & Torgan, 2010) and (Bica et al., 2012). This description, obtained from in vitro cultures of the axenic species, allowed for its taxonomic classification as *D. armatus*, according to Hegewald (2000).

Molecular identification and phylogenetic analysis

The ITS2 sequence of L60cmC1B-17519 presented a 100% percentage of identity with the species Scenedesmus armatus (Chodat) Chodat (JQ910904), *Desmodesmus sp*. (KJ680147) and *D. armatus* (MN759323). The phylogenetic tree analysis was performed to confirm the identification and analyze the evolutionary relationships of the studied microalgae with the species sampled in the GenBank database, as a second level of molecular identification. The L60cmC1B-17519 sequence is in the tree topology as a monophyletic group along with the *D. armatus* and *S. armatus* (Fig. 3). However, there was no conclusive result in the 18S topology since it did not align with the reference sequence.

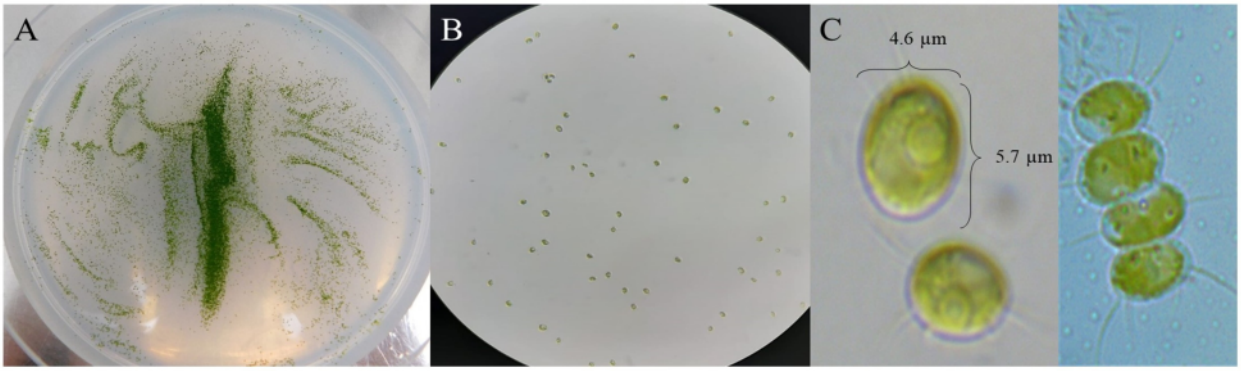

Fig 2. L60cmC1B-17519 culture. A. Petri dishes of the isolated microalgae. B. 40x magnification with the characteristic grouping of cells. C. 100x magnification of a cenobium of the microalgae, shape, grouping, and measurements.

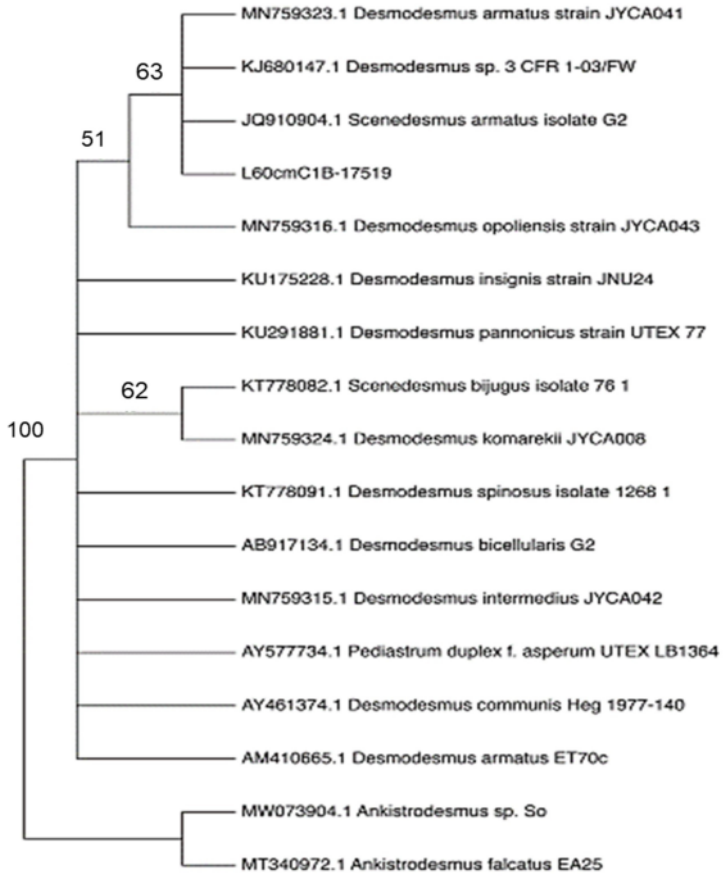

Fig. 3. Phylogenetic tree (Maximum likelihood with the nucleotide substitution model (K2 + G) based on ITS2 rDNA sequences. The bootstrap values (%) as statistical support of the clades are shown in the external nodes of the tree. The sequence is in the clade of *Desmodesmus armatus* (Chodat) E.H.

Algal growth

Cell density and dry biomass of L60cmC1B-17519 were monitored for 10 days, starting from 1.10E6 cells.mL-1 and 0.25 ± 0.21 g.L-1, respectively (Fig. 4A-4B). Growth of the microalgae is observed during the first eight days of culture until reaching a stationary phase from day nine. The final values of cell density and dry biomass were 1.88 ± 0.03E6 cells.mL-1 and 2.36 ± 0.03 g.L-1. These values are statis-

tically different from the baseline values ($p < 0.05$). The two determined parameters (cell density and biomass concentration) confirm a statistically significant growth of the microalgae during the culture time. The VP of the biomass was 0.24 g.L-1·d-1, and the SP was 0.10 d-1. For the isolated strain, the specific growth rate was 0.06 d−1. Duplication time for L60cmC1B-17519 was 11.22 d.

Nutrient removal

Nitrate, reactive phosphorus, and COD concentrations decreased progressively over the 10-day culture period (Fig. 5A-5C). A daily con-

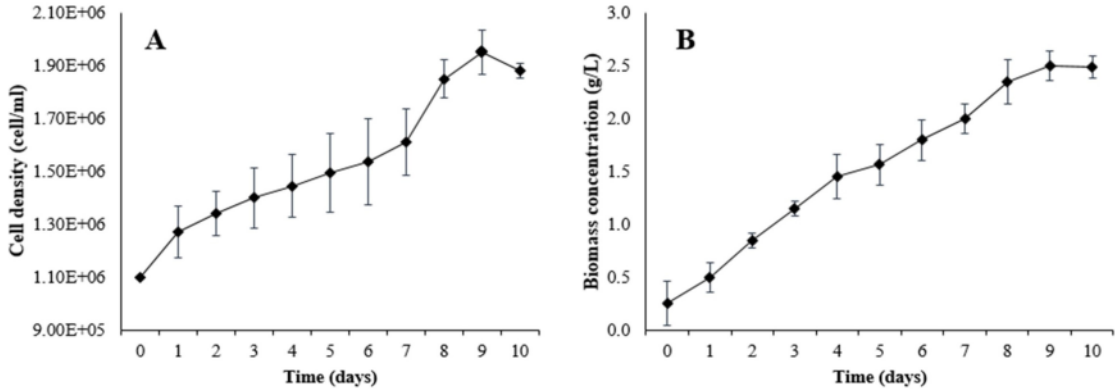

Fig. 4. Growth curve of L60cmC1B-17519 for ten days. A. Cell density (cells.mL-1) vs time (days). B. Biomass concentration (g.L-1) vs time (days). The values represent the mean ± standard deviation for three independent replicates for each condition.

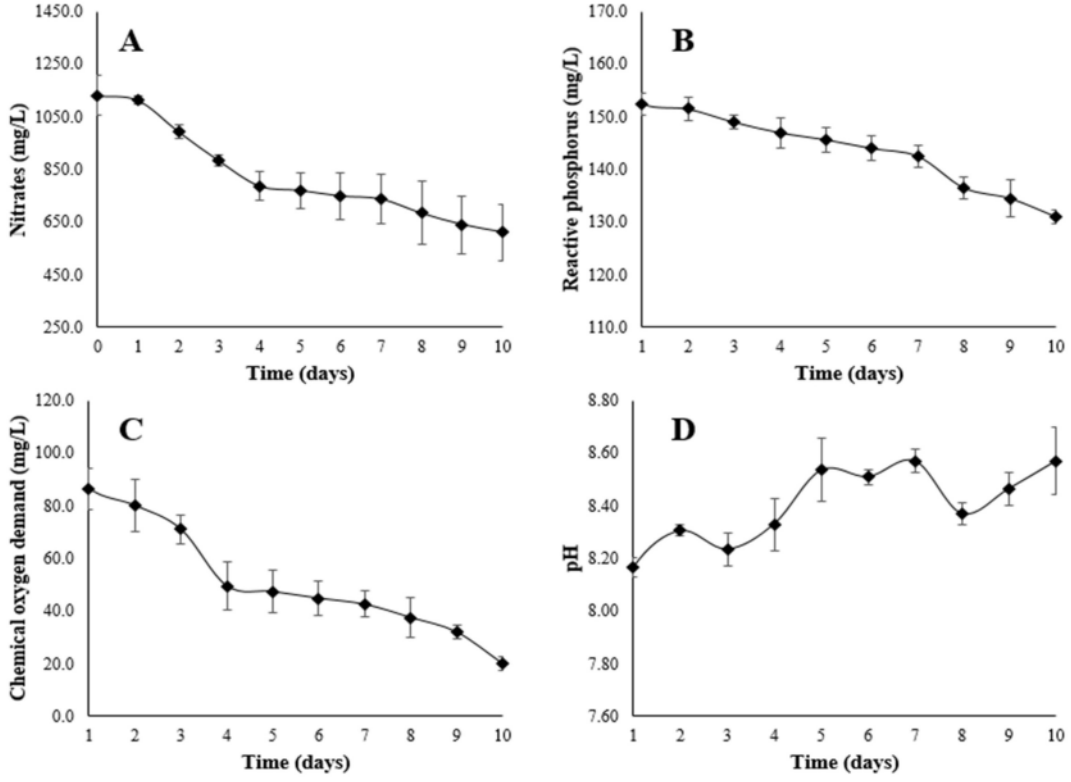

Fig. 5. Kinetics of nutrient removal. A. Nutrient removal of nitrates. B. Reactive phosphorus. C. Chemical oxygen demand. D. pH before neutralization. The values represent the mean ± standard deviation for three independent replicates for each condition.

trol was carried out with a 0.05 M HCl solution to keep a neutral pH. The pH of the medium, measured before neutralization, ranged between 7.80 and 8.50 during cultivation time (Fig. 5D).

The nitrate curve began on day 0 with a concentration of 1480.50 ± 4.95 mg.L-1 (NO_3^-), reaching 661.00 ± 35.36 mg.L-1 (NO_3^-) (Fig. 5A). The reactive phosphorus curve showed a significant decrease, with an initial value on day 0 of 44.50 ± 6.36 mg.L-1 (PO_4^{3-}) and a final value on day 10 of 14.10 ± 1.27 mg.L-1 (PO_4^{3-}) (Fig. 5B). The COD curve showed an initial value of 106.0 ± 4.24 mg.L-1 on day 0 and a final value of 20.00 ± 2.83 mg.L-1 on day 10 (Fig. 5C). Statistically, differences were observed ($p < 0.05$) for all the analyzed nutrients. Finally, the removal of NO_3^-, PO_4^{3-}, and COD were 55.35%, 68.31% and 81.13%, respectively.

Lipids determination

Total lipid content was quantified using wet and dry biomass, and the corresponding percentages are presented in Fig. 6. In wet extraction with chloroform:methanol (1:2), the percentage was 6.48% and with ethyl acetate 5.38%. For the dry extraction chloroform:methanol (1:2) the percentage was 21.50% and with ethyl acetate 17.34%. Although the results of the extractions with wet biomass did not show significant differences, in the dry extraction the best-extracting solvent was the mixture chloroform:methanol (1:2).

The lipid production in the wet biomass extraction was 0.013 g.L-1 using chloroform:methanol (1:2) and 0.016 g.L-1 with ethyl acetate. In the dry biomass extraction, lipid production was 0.054 g.L-1 and 0.043 g.L-1 with chloroform:methanol (1:2) and ethyl acetate, respectively. The VP and the SP for the best-extracting solvent on a dry basis (chloroform:methanol (1:2)) were 0.051 g.L-1.d-1 and 0.021 d-1, respectively.

FFA determination

FFA extraction experiments were performed using high lipid content samples from the lipid procedures with the mixture chloroform:methanol (1:2) and ethyl acetate. The FFA were quantified from dry biomass using chloroform:methanol (1:2) and ethyl acetate, and the resulting percentages are shown in Fig. 7. In the extraction with chloroform:methanol (1:2), the percentage of FFA was 52.80% and in ethyl acetate 39.20%. However, no statistically significant differences were found for the FFA extractions.

Discussion

In this study, we successfully isolated and identified the Ecuadorian strain L60cmC1B-17519 as *D. armatus* and evaluated its biotechnological potential for bioremediation and biofuel-related lipid production. Morphological identification of the L60cmC1B-17519 strain was

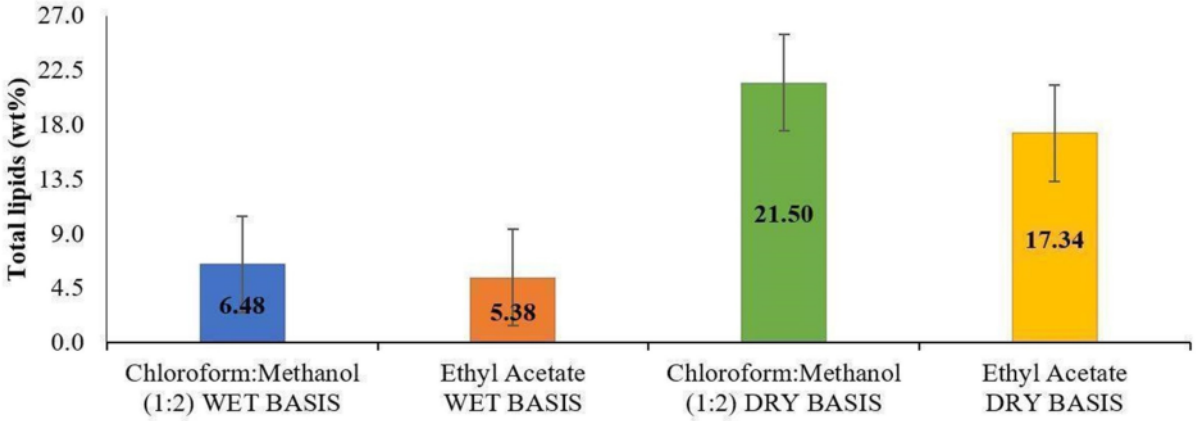

Fig. 6. Percentages of the extractions on a wet and dry basis using the mixture chloroform:methanol (1:2) and ethyl acetate. The values represent the mean ± standard deviation for three independent replicates for each condition.

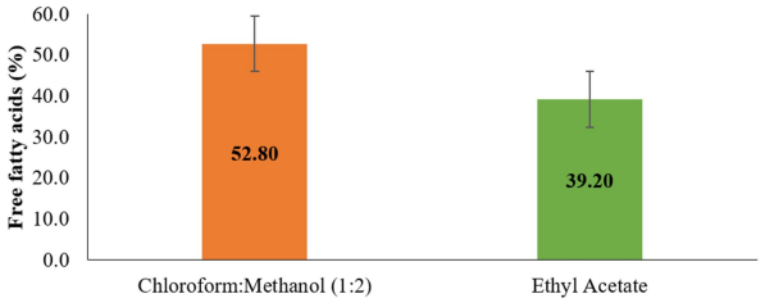

Fig. 7. Percentage of FFA extracted from L60cmC1B-17519 using chloroform:methanol (1:2) and ethyl acetate. The values represent the mean ± SD for three independent replicates for each

made with comparisons with species described in several bibliographic resources, finding similarities with *D. armatus*. The genus *Desmodesmus* originated from *Scenedesmus* and differs morphologically by the presence of spines at the terminal and intermediate ends of the cells (Geraldo et al., 2015). In the molecular identification, the phylogenetic tree with the ITS marker showed the homology between L60cmC1B-17519 and the NCBI *D. armatus* species, corroborating the morphological identification. However, the marker 18s showed no conclusive results. This may be associated with the level of conservation of the 18S region, which could be highly conserved for this taxonomic group, unable to differentiate *D. armatus*. On the other hand, the ITS region exhibits enough variability (Lortou & Gkelis, 2019) to distinguish the species in this study, enabling molecular identification.

Desmodesmus spp. is very common in any body of water, whether oligo, meso, or eutrophic, but mainly in the latter environment. They are also indicators of the degree of contamination of these systems that influences their current use (Sarfraz et al., 2023). In this study, the strain cataloged as *D. armatus* was isolated from the Limoncocha Lagoon, which represents a suitable habitat for this microalga due to the climatic characteristics of the area and the anthropogenic activities that occur in its surroundings (Hentschke & Torgan, 2010; Huang et al., 2012). Proper identification of microalgae and microorganisms, in general, is essential for conducting tests of biotechnological potential, maintaining a record of species present in Ecuador, and accurately comparing performance across various indicators, such as nutrient removal and lipid generation, with other algae of similar or different genera and/or species.

Microalgal growth is one of the most important factors to consider when evaluating their biotechnological potential. It is largely determined by the availability of nutrients in the medium, its conditions (light, temperature, pH, etc.), and the initial cell density. In general, the species L60cmC1B-17519 shows a biomass generation behavior similar to other studies with microalgae of the same and different genus. Studies such as the one described by Guldhe et al. (2017) report that in a 30-day culture in BG-11 medium with temperature control at 28 °C, light intensity 44.5 µmol.m-2.s-1. and photoperiod of 14:10 h, *D. armatus* generated a biomass concentration of 2.58 g.L-1. Additionally, Okpozu et al. (2019) reported that in a 14-day culture in BBM medium, *D. armatus* generated a biomass concentration of 0.28 g.L-1.

Research on cultures of *Scenedesmus sp.*, in BG-11 medium, adding pulses of CO_2 (96:4) as a carbon source, showed a maximum cell density and dry biomass of 5.7 ± 0.9E107 cells.mL-1 and 2.92 ± 0.45 g.L-1, respectively (Brennan & Owende, 2010). On the other hand, the biomass VP and the biomass SP in this study were 0.24 g.L-1.d-1 and 0.10 d-1, respectively. The specific growth rate (µ) was 0.06 d-1, and the duplication time (tD) was 11.22 d. Okpozu et al. (2019) reported a biomass VP of 0.02 g.L-1 d in the species *D. armatus*, in a BBM medium at 30 °C for 14 days. Similarly, Sun et al. (2019) reported productivity of 1.95 g.L-1 d at 25 °C for 11 days, adding CO_2 pulsations. Encalada-Rosales et al. (2022) reported a specific growth rate of 0.08 d-1 and a duplication time of 8.54 d for *Desmodesmus communis* (E. Hegewald) E. Hegewald. Even though these values are higher than those presented in this study, they obtained a VP of 0.22 g.L-1 d and a final biomass concentration of 2.19 g.L-1. It is important to highlight that additional modifications to the culture medium, such as applying a CO_2 source, can positively influence the growth and biomass generation of microalgae.

Microalgae cultivation has significant short-term applications in bioremediation, efficiently removing nutrients such as nitrogen, phosphorus, and carbon from water bodies, making them suitable for phytoremediation processes (Bhardwaj et al., 2020; Qin et al., 2023).

Various studies have demonstrated the bioremediation potential of species like *Scenedesmus sp.* and *Chlorella vulgaris* Beijerinck in wastewater treatment. For instance, *C. vulgaris* has been shown to remove 97% of nitrates and 89% of orthophosphates in pig slaughterhouse wastewater after 13 days of culture (Medrano-Barboza et al., 2021), while *Scenedesmus sp.* and *C. vulgaris* present high rates of nutrient removal (above 80%) for wastewater treatment (Medrano-Barboza et al., 2022). Similarly, *C. vulgaris* exhibited 86% removal of inorganic nitrogen and 70% of inorganic phosphorus in municipal wastewater over 14 days (Lau et al., 1996). In our study, L60cmC1B-17519 demonstrated comparable bioremediation potential, suggesting that *D. armatus* holds promise for application in biotechnological processes, including the bioremediation of contaminated water, and merits further investigation.

Microalgae can accumulate large amounts of lipids in their cells, making them an attractive alternative renewable energy source (Sun et al., 2019). However, the production costs nowadays are very high. For this reason, using wet biomass is an alternative to reduce costs associated with drying or freeze-drying processes traditionally used in the use of microalgal biomass (Qin et al., 2023). In our study, the best extraction result was 21.50% (0.054 g.L-1), obtained with the chloroform:methanol (1:2) on a dry basis. The lipid yield reported in another study using a similar microalgal species, *Desmodesmus subspicatus* (Chodat) E. Hegewald & A.W.F. Schmidt, was 29% when extracted with a chloroform:methanol (2:1:0.8) solvent system under nitrogen-limited conditions (Chaudhary et al., 2017). On the other hand, Sun et al. (2019 reported that *Desmodesmus sp*. cultured in BG11 medium for 35 days with temperature control of 25 °C, obtained 26.64% of lipids extracted using chloroform:methanol:water (1:2:0.8). Both studies used *Desmodesmus spp*. microorganisms suggest that modifying the culture duration and optimizing the culture medium may offer potential benefits for increasing the lipid yield of microalgal species, which can also be applied to Desmodesmus armatus.

Microalgal lipids contain free FFA, which are essential for producing sustainable biodiesel. It is important to use microalgae with high FFA productivity as a raw material since biodiesel is composed of fatty acid methyl esters, triacylglycerols, diacylglycerols, free fatty acids, and phospholipids, all of which are found in lipids (Krohn et al., 2011; Chen et al., 2012). Therefore, the FFA percentage is a critical factor in biodiesel production. Chen et al. (2012 reported that *Scenedesmus sp.* cultured in BG-11 medium with compressed aeration (1% CO_2) for ten days generated an FFA value of 70.3% when extracted with chloroform (1:2), after storing the biomass for one day at 25°C and lyophilizing it at -80°C. In the present study, *D. armatus* yielded 52.80% FFA with chloroform (1:2) and 39.20% with ethyl acetate. Although these values differ significantly from those reported in the literature, the potential of *D. armatus* (L60cmC1B-17519) for biofuel production should not be dismissed without considering possible treatments for the biomass and the conditions of the culture medium.

Nutrient deficiency and stress are commonly applied to achieve high lipid concentrations in microalgal cultures. Most lipid regulation strategies involve reducing nitrogen concentration by 50% of the initial level (Park et al., 2011; Sun et al., 2019). Additionally, physical strategies include altering carbon dioxide concentration, temperature, and light intensity to enhance biomass and lipid production. Other approaches involve genetic engineering and metabolic techniques. These modification strategies vary depending on the intended purpose of the culture, whether for biodiesel production, supplement creation, or other applications.

Conclusions

In this study, *Desmodesmus armatus* (L60cmC1B-17519) was identified and evaluated for its potential as a raw material for biofuel production and bioremediation. The removal of nutrients, including nitrates, reactive phosphorus, and COD, suggests that this species can be utilized in bioremediation processes, presenting an alternative for biotechnological applications. Lipid and FFA percentages showed better results when extracted from dry biomass using the chloroform solvent system than ethyl acetate. Although this strain of *D. armatus* exceeded the required lipid content for biofuel production, the FFA percentage was below the necessary threshold for its classification as a source microalga for biofuels. Nevertheless, its use in biotechnological applications cannot be ruled out, and further studies are recommended.

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