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Pilot-scale phycoremediation using *Muriellopsis* sp. for wastewater reclamation in the Atacama Desert: microalgae biomass production and pigment recovery

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ABSTRACT

Municipal wastewater phycoremediation represents a promising circular economy-based process for wastewater reclamation used to recover water and produce biomass. This study aimed to evaluate a pilot-scale phycoremediation system, using the most efficient strain of microalgae for wastewater reclamation in the Atacama Desert. Nitrogen and phosphorus removal, as well as biomass growth, were compared in different microalgae treatments, namely *Muriellopsis* sp., *Scenedesmus almeriensis*, *Chlamydomonas segnis*, *Chlorella pyrenoidosa* and *Chlorella vulgaris*. The most efficient treatments, *Muriellopsis* sp. and *S. almeriensis,* were scaled up to 20-L bubble column reactors to evaluate nutrient removal and biomass biochemical profile for potential biotechnological application. Finally, *Muriellopsis* sp. was selected for a pilot-scale phycoremediation experiment (800-L raceway), which removed 84% of nitrogen, 93% of phosphorus and other chemical compounds after 4 days of treatment to meet most of the Chilean standards for irrigation water (NCh. 1333. DS. MOP No. 867/78). Faecal coliforms count was reduced by 99.9%. Furthermore, biomass productivity reached 104.25 mg·L^{-1}·day^{-1} value with 51% protein, and pigment content of 0.6% carotenoid, with 0.3% lutein. These results indicate the potential of wastewater phycoremediation at an industrial scale for the production of irrigation water and carotenoid using *Muriellopsis* sp.

Key words | irrigation water, lutein, *Muriellopsis* sp, phycoremediation, pilot-scale treatment, wastewater

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HIGHLIGHTS

- Phycoremediation treatment by *Muriellopsis* sp. reduces nutrient concentrations while increasing biomass at a pilot scale in outdoor conditions.
- *Muriellopsis* sp. treatment completely removes faecal coliform, meeting Chilean irrigation water standards for water reclamation.
- Produced biomass has high carotenoid and lutein content.

INTRODUCTION

Municipal and industrial wastewater production increases with urban and rural populations. Their discharge to the environment contributes to increasing highly toxic compounds, unknown levels of pathogens, hydrocarbons, nutrients, toxins, organic and inorganic matter, and endocrine disruptors that deteriorate the ecosystem and accelerate eutrophication (Moretti *et al.* 2019). In many developing countries, wastewater treatments are expensive, energy-intensive, unsustainable or have low yield in reusable water (Abinandan *et al.* 2018). Indeed, by the year 2050, safe water supply and sanitation services will face unprecedented challenges;

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however, water problems represent a global concern with local solutions.

The Atacama Desert, the aridest region in the world, is considered a water hotspot due to scarcity of freshwater supply and is also threatened by industrial mining activities (Saavedra *et al.* 2018). The potable water for its major coastal city comes from a desalination plant, and the resulting municipal wastewater is discharged back to the environment with almost no treatment at all. Water reclamation in this urban area could aid in reducing water withdrawal, supplying water for irrigation purposes and reducing the environmental impact related to effluent discharge. The treatment of wastewater through algal intervention is known as phycoremediation and has been studied for over 60 years. It has proven to be an efficient technique for algal growth as well as for value-added product development (Hu *et al.* 2018). Some advantages over traditional wastewater treatments include high levels of nitrogen and phosphorus recovery from effluents and solid wastes (Cuellar-Bermudez *et al.* 2017) with low energy consumption, zero carbon footprint and without utilizing chemicals for sludge formation. It is also an alternative process for microalgae production that reduces the environmental impact compared to systems that use freshwater and fertilizer (Agüera *et al.* 2020). At a global scale, nutrient recovery technologies are quickly enhancing product value chain and decreasing water depletion, allowing to build a sustainable society through the circular economy model (Robles *et al.* 2020). The Atacama Desert coastal area has excellent conditions to carry out phycoremediation and microalgae production due to land availability, moderate temperature and high solar radiation. Under such conditions, municipal wastewater phycoremediation could reduce local water withdrawal for irrigation purposes. Several experimental evidences underline the effectiveness of microalgae for phycoremediation or high-value product development such as proteins, lipids and pigments, in strains belonging to *Muriellopsis* (Blanco *et al.* 2007; Martínez *et al.* 2018), *Scenedesmus* (Cerón *et al.* 2008; Li *et al.* 2011; Jebali *et al.* 2018), *Chlamydomonas* (Saavedra *et al.* 2018) and *Chlorella* (Wang *et al.* 2010; Lu *et al.* 2015) genera. Moreover, raceway (RW) reactor technology is extensively used in microalgae culture (Acién *et al.* 2017) and comparable to open ponds and stabilization tanks, the most globally used wastewater treatment technology (Verbyla *et al.* 2016). However, nutrient recovery technology for high-value product production still needs further research to guarantee large-scale efficiency and product safety (van der Spiegel *et al.* 2013).

This study aimed to evaluate the effectiveness of different microalgae commonly used for the removal of nutrients from local Primary Treatment Wastewater (PTWW). Additionally, the technology was scaled up to pilot-size RW photobioreactors, using the most efficient strain to meet irrigation water quality standards, as well as to characterize the resulting biomass to search for high-value products such as pigments.

METHODS

Microalgae strains and culture medium

Microalgae strains used for phycoremediation namely, *Muriellopsis* sp. (MCh), *Scenedesmus almeriensis* (SA), *Chlamydomonas segnis* (CHY), *Chlorella pyrenoidosa* (CP) and *Chlorella vulgaris* (CV), were provided by the microalgae collection of the Applied Microbiology Unit of the Centre for Bio-innovation, University of Antofagasta (UMA-CBIA, UA), Chile. Microalgae strains came from the isolation of MCh, CHY, CP and CV from local freshwater deposits, whereas SA came from a commercial strain.

Microalgae were kept inactive in the UMA5 culture medium ($0.40\,g{\cdot}L^{-1}$ $NaNO_3$, $0.03\,g{\cdot}L^{-1}$, NaH_2PO_4, $0.17\,g{\cdot}L^{-1}$ $NaHCO_3$, $0.08\,g{\cdot}L^{-1}$ Zn, $0.90\,g{\cdot}L^{-1}$ Mn, $0.03\,g{\cdot}L^{-1}$ Mo; Winkler, Santiago, Chile), in a temperature-controlled room at 20 °C and constant low-radiation ($300\,\mu mol{\cdot}m.^{-2}s^{-1}$) using fluorescent tubes (Osram L 36 W/7635 Munich, Germany) and indirect lightning, until further experimentation.

Phycoremediation treatments were assayed in UMA-CBIA facilities, located in the Atacama Desert major coastal city, Antofagasta, Chile (23° 42′07.0″S 70° 25′22.8″W). PTWW used for phycoremediation treatments was provided by a local sewage treatment plant, SEMBCORP-Aguas del Norte SA, located within the city. Municipal raw wastewater pretreatment consisted of metal bars and a wide mesh to remove the floating debris, followed by a degritting and degreasing unit, which reduces flowrate to settle sands and float greases. PTWW was pumped and collected from the primary treatment unit entrance and transported promptly to UMA-CBIA facilities for immediate use in phycoremediation systems. The PTWW composition is typical of sanitary sewage water from the area (Supplementary Table S1); however, initial nutrient analyses were required for every experiment because values were variable during monitoring campaign (Supplementary Table S2).

Phycoremediation trials

Most efficient strain

Each microalga was inoculated in 2-L round bottom glass culture flasks containing 1-L PTWW. Flasks were kept under 20 °C in a temperature-controlled room and direct lightning (83 μmol·m^{-2}·s^{-1} average radiation) from two fluorescent lamps (Osram L 36 W/7635 Munich, Germany), 24:0 photoperiod and constant aeration (0.4 L·min^{-1}). A nutrient baseline (NBL) control using PTWW with no microalga inoculum was included. Nutrient removal and biomass increase were assessed to select the most efficient strain.

Bubble column reactor trial (20 L)

Microalgae treatments were scaled up to 20-L bubble column reactors under natural outdoor conditions to validate the most efficient strain performance. MCh and SA strains were inoculated at 0.4 g·L^{-1} in a 25-L column reactor. These systems used transparent, conic base acrylic cylinders with a 25 cm diameter and a height of 1.10 m. Airflow was bubbled up from the bottom at a range of 10–15 L·min^{-1} to agitate and remove dissolved oxygen (DO). Reactors were kept in an outdoor facilities, under natural temperature (26 °C average) and sunlight conditions 12:12 photoperiod, 2,000–2,500 μmol·m^{-}2·s^{-1} irradiance during summer), constant airflow and no pH control for 4 days treatment. The NBL control system was also included. All experiments were assayed in triplicate.

Raceway trial 800 L (RW)

To scale up nutrient removal in pilot conditions, MCh was cultured in a 3.8 m^2 area and 0.2 m water column fibreglass raceways (800 L). For experimental conditions, PTWW systems were inoculated with 0.3 g·L^{-1} MCh and incubated for 4 days in the same conditions as the bubble column reactor trial. DO was measured once a day (at 12:00), during maximum metabolic activity, using a multiparameter waterproof meter (Hanna Instruments, Romania). Two control systems included were the NBL control and microalgae growth control using UMA5 as a culture medium (UMA5 control). All experiments were run in triplicate. The photosynthetic efficiency was measured using an AquaPen-C AP-C 100 fluorometer (Photon Systems Instruments, Czech Republic) to control the physiological conditions of microalgae. The microalgal sample was incubated in the dark for 5 min to ensure all reaction centres were oxidized. Further, the device creates a saturation pulse that induces chlorophyll fluorescence, thus reflecting the real photosystem response of the microalgae during its culture condition (Strasser *et al.* 2000).

Nutrient removal analysis

Water samples were collected from the experimental systems every 1 or 2 days, in triplicate. Each sample was filtered through a 1.6-μm filter (Munktell Filter AB, Falun, Sweden), collected in a glass bottle and immediately analyzed.

Ammonium concentration was determined using the Berthelot reaction (Krom 1980). The absorbance was read at 690 nm on a Halo RB-10 spectrophotometer (Dynamica Scientific Ltd, Newport Pagnell, UK). Nitrate was also measured spectrophotometrically at 220 and 275 nm (following the procedures described in Karlsson *et al.* 1995). Additionally, phosphorus measurement was based on phosphorus–vanadate–molybdate colourimetry at 882 nm, as described in Standard Methods (1999). Nitrogen content was outlined as the sum of nitrogen from ammonium and nitrate content ($N–NH_4^+ + N–NO_3^-$), whereas phosphorus was expressed as the subtraction of phosphorus from phosphate ($P–PO_4^{3-}$) concentration.

Biomass biochemical analysis

Microalgae biomass concentration was calculated as the difference between total dried sample weight (including filter) and dried filter weight. Briefly, the sample (100–200 mL) was passed through a 1.6-μm fibreglass filter (Munktell Filter AB, Falun, Sweden) and dried for 24 h at 105 °C. Biomass samples for biochemical analyses were recovered at the end of each experiment by centrifugation at 76.6 Hz for 15 min (Rotanta 460 R, Hettich Zentrifugan, Tuttlingen, Germany). Samples were also frozen at –80 °C and lyophilized to obtain 1 g of biomass for further analyses. Ash content (non-organic matter) was quantified, calcining 0.5 g lyophilized biomass at 540 °C for 2 h (Liu *et al.* 2015). Data were expressed as an ash-free percentage.

Total protein content was determined using the modified Lowry method (López *et al.* 2010). Sodium hydroxide was added to 15 mg lyophilized samples and heated at 100 °C. Once cooled, sodium hydroxide, water and reagent A (copper sulfate, sodium-potassium tartrate, and sodium carbonate) were added. After adding 50% Folin–Ciocalteu reagent, absorbance was measured at 750 nm, using bovine serum albumin (Sigma Chemical Co., St. Louis, Mo) as the standard. Lipid content was determined using 100 mg lyophilized sample ground with alumina, followed by several rinses in chloroform-methanol (2:1) (Merck, AG, Darmstadt, Germany) and centrifugation at 46.6 Hz (Kochert 1978). Subsequently, hydrochloric acid (HCl) and magnesium chloride were added to the sample and centrifuged. The organic phase was recovered and dried under a nitrogen column. The total lipid content was determined as the percentage of dried biomass. Carbohydrates were determined by the difference out of a hundred after subtracting lipids, proteins and the ash content.

Total carotene content was performed based on the method described by Cerón *et al.* (2008). Briefly, the lyophilized sample was ground with alumina (1:1). A saponification step was included by adding a potassium hydroxide, water, ethanol, and hexane solution. The liquid fraction was collected after centrifugation at 46.6 Hz and dried under a nitrogen column. The sample was further diluted in acetone, and carotene absorbance was measured at 444 nm. Additionally, 1 mL of carotenoid solution was filtered through a 0.22-μm filter and frozen at –20 °C for further analysis. Lutein extraction and measurement were performed according to Cerón *et al.* (2008) methodology. The method included a cell disruption, a chemical alkaline treatment and a solvent extraction that allowed a high percentage of lutein recovery from the microalgae biomass. The high-performance liquid chromatography procedure to determine lutein content used water/methanol (2:8, v/v) and acetone/methanol (1:1, v/v) as eluents. Lutein was quantified by integration at 450 nm using a standard solution (Sigma Chemical Co., St. Louis, MO).

Water quality analysis for irrigation purposes

Water analyses were based on the parameters established in the Chilean regulation for the use of irrigation water (NCh. 1333), and performed at the Environmental Laboratory SGS Chile Ltd, following the Standard Methods (1999) (Supplementary Table S3), and validated by the National Normalization Institute.

Biological analysis

Faecal coliforms were detected using the Most Probable Number (MPN) method. Each serially diluted sample was inoculated in a lactose broth (Thermo Fisher Scientific, Waltham, MA, USA) incubated at 35 °C for 48 h, according to the Standard Methods for the examination of water and wastewater (1999). Presumptive gas positive dilutions were further inoculated in a brilliant green bile broth (Thermo Fisher Scientific, Waltham, MA, USA) and incubated at 44.5 °C to determine the faecal coliforms. Calculus for MPN was based on the proportion of confirmed gassing tubes for three consecutive dilutions.

Statistical analysis of data

Statistical analyses were performed using Statgraphics Centurion XIII software (Statgraphics Technologies, Inc. VA, USA). Differences between control and treatment groups were determined using Student's t-test. Multiple treatments were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test (multiple comparisons). The statistical differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Most efficient strain

In the 1-L system trial, nitrogen concentration decreased in all microalgae treatments from 43.3 $mg{\cdot}L^{-1}$ to values under 3.5 $mg{\cdot}L^{-1}$ at day 12 (Figure 1(a)). Additionally, phosphorus concentration decreased from 7.2 $mg{\cdot}L^{-1}$ to under 3.7 $mg{\cdot}L^{-1}$ in CP and CV treatments, whereas MCh, SA and CHY reached values close to 1 $mg{\cdot}L^{-1}$ at day 12 (Figure 1(b)). Control values decreased significantly due to native microalgae growth and nutrient consuming bacteria. The MCh strain had the most efficient performance, with the lowest nutrient values throughout the experiment, removing 99% of nitrogen, and 94% of phosphorus. The SA treatment removed 96% of nitrogen and 87% of phosphorus. Although CV and CP treatments showed high nitrogen removal, there was no difference in the phosphorus percentage compared to the NBL control value (close to 50%) (Figure 1(c)). The MCh biomass productivity performed significantly higher (33.8 $mg{\cdot}L^{-1}{\cdot}day^{-1}$) than other strains, followed by SA (10.7 $mg{\cdot}L^{-1}{\cdot}day^{-1}$) and CHY (10 $mg{\cdot}L^{-1}{\cdot}day^{-1}$), while CV and CP showed the lowest biomass productivity (6.8 $mg{\cdot}L^{-1}{\cdot}day^{-1}$ and 4.7 $mg{\cdot}L^{-1}{\cdot}day^{-1}$, respectively) (Figure 1(c)). All five species were able to acclimatise and grow in local PTWW without additional nutrients–MCh and SA were the most efficient and *Chlorella* strains the less efficient in nutrient remova. Similar to our results, *Scenedesmus obliquus* has been previously reported to show higher potential for removing nutrients from filtered raw sewage, reaching values close to 99% of nitrogen and 98% of phosphorus, compared to *Chlorella sorokiniana* which was able to remove 86% of nitrogen and 68% of phosphorus (Gupta *et al.* 2016). Additionally, *Scenedesmus* was more efficient than *Chlorella* in agroindustry

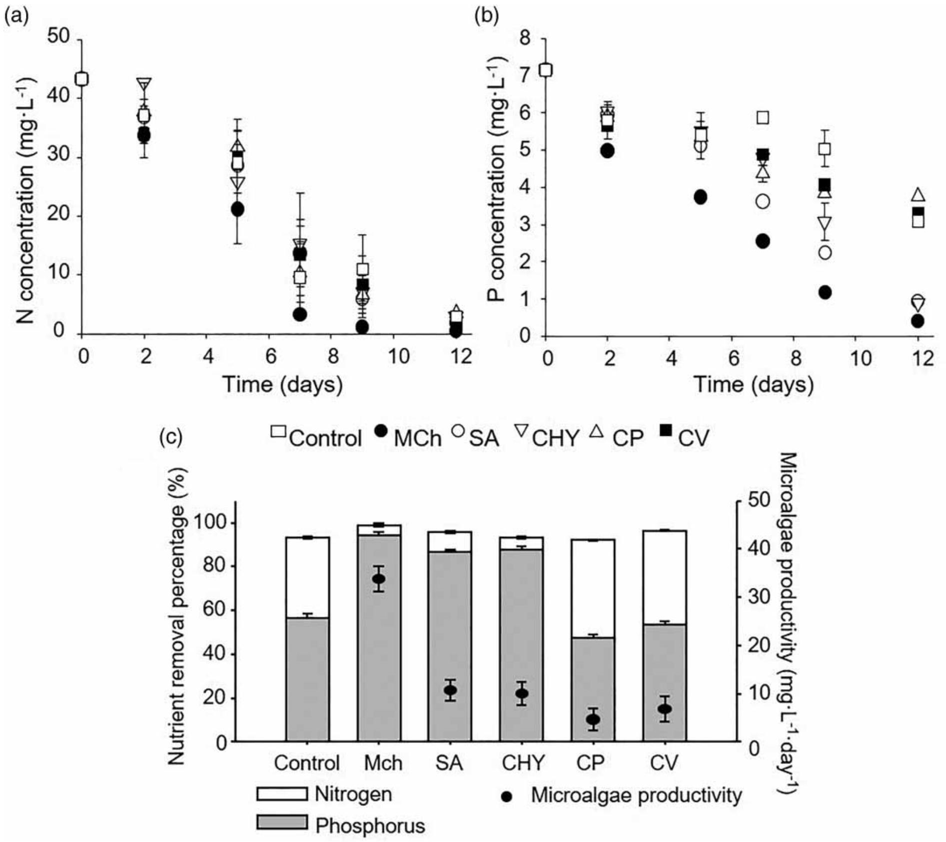

Figure 1 | Microalgae performance in 1-L PTWW phycoremediation systems for 12 days. (a) nitrogen concentration ($N\text{-}NH_4^+ + N\text{-}NO_3$). (b) phosphorus concentration ($P\text{-}PO_4$). Symbols represent different microalgae treatments: bold square, nutrient baseline control (control); black circle, *Muriellopsis* sp. (MCh); bold circle, *S. almeriensis* (SA); bold inverted triangle, *C. segnis* (CHY); bold triangle, *C. pyrenoidosa* (CP); black square, *C. vulgaris* (CV). Error bars denote differences among treatments after the ANOVA test ($p < 0.05$). (c) Nutrient removal percentage. White bar represents nitrogen concentration ($N\text{-}NH_4^+ + N\text{-}NO_3^-$), whereas grey bars represent phosphorus concentration ($P\text{-}PO_4^{3-}$). Black circles denote microalgae productivity. Error bars denote differences among treatments after the ANOVA test ($p < 0.05$).

wastewater (González *et al.* 1997). However, *Chlorella* species are widely used as wastewater treatments because they show great abilities in nutrients and contaminant removal (Wang *et al.* 2010). There is evidence that shows that the microalgae ability to adapt to the harsh conditions in wastewater are species-specific (Posadas *et al.* 2017). Some of them can acclimatise more efficiently to stress than others (Osundeko *et al.* 2014). Taken together, these results suggest that MCh and SA strains have acclimated better to local conditions than *Chlorella* strains.

MCh and SA had the most potential to produce higher biomass concentration during phycoremediation. These results showed a $33.8\,mg{\cdot}L^{-1}{\cdot}day^{-1}$ productivity in MCh treatment without the addition of any nutrient, indicating that local PTWW has enough nutrients to sustain microalgae growth during 12 days of phycoremediation. Furthermore, MCh productivity was almost four times higher than SA and almost nine times higher than *Chlorella* strains under local conditions. Several microalgae genera have been reported for efficient growth during phycoremediation, and *Oscillatoria*, *Scenedesmus*, *Chlorella* and *Nitzchia* are ranked as the most pollution-tolerant microalgae (Oberholster *et al.* 2019). Similar growth rates have been reported of *Chlorella* and *Scenedesmus* strains in wastewater, reaching higher productivity biomass values compared to other microalgae genera (Ferro *et al.* 2018). Moreover, MCh can be cultured in a specific culture medium with up to 66% secondary treatment wastewater without decreasing biomass productivity and reaching $0.5\,g{\cdot}L^{-1}{\cdot}day^{-1}$, 38% lower than using only a specific culture medium (Gómez *et al.* 2013). These results highlight the importance of selecting microalgal species according to their adaptability to local conditions, and thus extend their applicability for wastewater treatment.

Bubble column reactors (20 L)

MCh and SA were selected as the most efficient strains for scaling up treatment volume to 20 L and outdoor conditions.

Both treatments decreased the nitrogen from 56 mg·L^{-1} to 8.6 mg·L^{-1} (84%) and 13.5 mg·L^{-1} (97%) concentration, respectively (Figure 2(a)). Phosphorus concentrations showed a significant decrease from the initial value of 6 mg·L^{-1} to 0.8 mg·L^{-1} (75%) concentration in MCh treatment on day 2 and 0.7 mg·L^{-1} (98%) in SA treatment by day 4 (Figure 2(b)). NBL control conditions with no inoculum removed up to 9% of nitrogen and 45% of phosphorus, suggesting that factors other than assimilation by microalgae might also play a role during treatment.

Delgadillo-Mirquez *et al.* (2016) results show the impact of microbial respiration, nitrification and abiotic losses (nutrient stripping and precipitation) during wastewater treatment, showing that up to 50% of total nitrogen was assimilated by microorganisms. Our results suggest nutrient uptake by indigenous PTWW bacteria or indigenous microalgae could have led to phosphate reduction because, after 6 days of treatment (data not shown), an unknown microalga grew in the NBL control system, which could have consumed all the nutrients.

Microalgae was able to augment biomass concentration during treatment to 762 mg·L^{-1} MCh and 640 mg·L^{-1} SA in 4 days (Figure 2(c)). Moreover, microalgae productivity reached 109 mg·L^{-1}·day^{-1} and 71.6 mg·L^{-1}·day^{-1}, respectively (Figure 2(d)). No growth was observed in the control group in 4 days of treatment. These results demonstrate the effectiveness of MCh and SA remediation at laboratory scale and natural outdoor conditions, as a precursor to scaling up to pilot scale. It is essential to demonstrate strain effectiveness by scaling up volume and including outdoor conditions, in order to minimize production costs at pilot or industrial scale. Many studies report successful phycoremediation trials in small laboratory-scale experiments (flask, bottles and small reactors) and light-controlled conditions with highly effective rates of nutrient removal (Cuellar-Bermudez *et al.* 2017; Posadas *et al.* 2017). For example, remediation treatments using *C. vulgaris*, *Chlorella kessleri* and *S. obliquus* strains yield higher nitrogen removal (over 95%) in 200-mL flasks and controlled light conditions (Álvarez-Díaz *et al.* 2017). Other treatments, using enriched microbial biomass inoculum in 200-mL bottles reported complete phosphate removal by day 6 (Delgadillo-Mirquez *et al.* 2016). Phycoremediation treatments in volumes similar to our study report *Chlorella* sp.

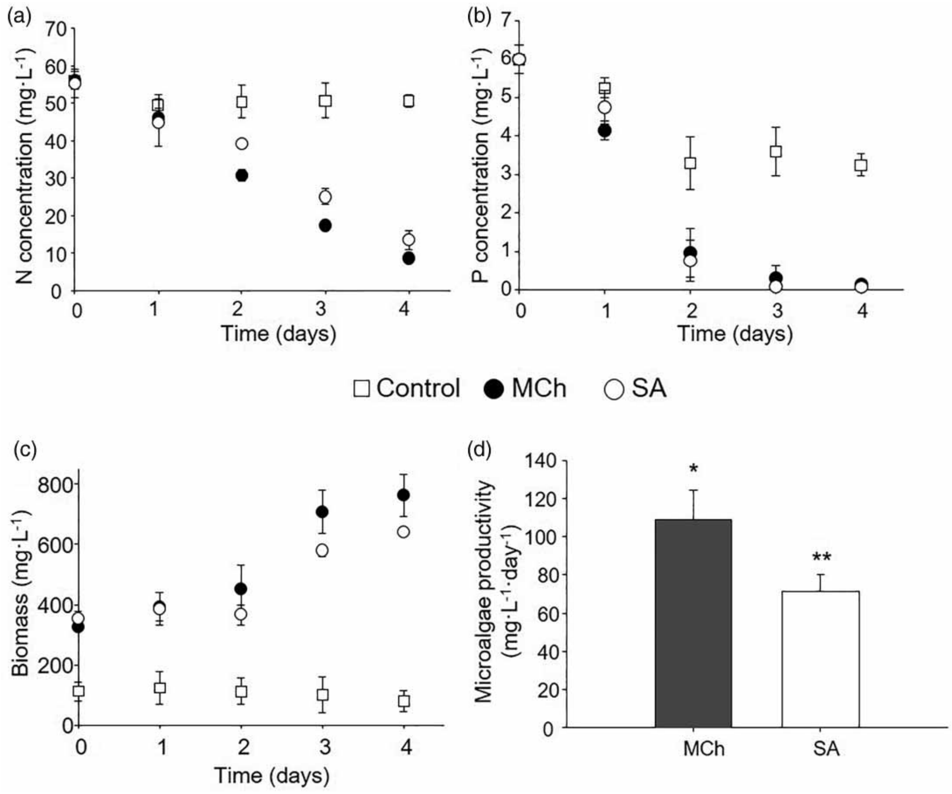

Figure 2 | Performance of microalgae in 20-L PTWW phycoremediation systems for 4 days. (a) Nitrogen concentration (N-NH$_4^+$ + N-NO$_3^-$). (b) Phosphorus concentration (P-PO$_4^{3-}$). (c) Microalgae biomass growth. Symbols represent different microalgae treatments: see Figure 1 for abbreviation details. (d) Microalgae productivity rate. Error bars denote differences among treatments after the ANOVA test ($p < 0.05$). Asterisks denote significant differences after Student's t-test ($p < 0.05$).

as an effective treatment in nutrient uptake from autoclaved and raw centrate. Nutrient removal showed values close to 89% of total nitrogen and 80.9% of total phosphorus during the first 4 days of treatment. Conditions included 25-L coil reactors and controlled light conditions designed to maximize photosynthesis rate (Li *et al.* 2011). Our results validate the effectiveness of both strains in nutrient removal and microalgae growth, suggesting that PTWW had sufficient nutrients to sustain both microalgae growth and that MCh, as well as SA, adapted successfully to grow in PTWW and the Atacama Desert coastal outdoor conditions.

At the end of phycoremediation treatments, MCh and SA showed higher protein content (>50%) than previously reported (<30% MCh, <50% SA), whereas lipid (10–16%) and carbohydrate content (25–33%) were similar to microalgae grown in specific culture medium (Gómez *et al.* 2013). Furthermore, MCh lutein content (0.3%) was consistent with previous reports (Del Campo *et al.* 2000). In contrast, SA lutein percentage (0.3%) was lower compared to microalga grown in specific culture medium (0.5%) (Del Campo *et al.* 2000) (Supplementary Figure S1). It has been previously reported that there is a correlation between wastewater tolerance and carotene pigment accumulation in microalgae as a stress response (Osundeko *et al.* 2014). These results suggest that PTWW has sufficient nutrients to produce valuable products in both strains and that pigment accumulation might be considered as a stress response to wastewater environment.

Pilot-scale raceway culture systems (800 L RW)

Only MCh was used for PTWW phycoremediation in the 800-L raceway system to scale up the experiments to pilot size volumes. This strain was selected over SA due to high nutrient removal and significantly higher productivity in 20-L trials. Furthermore, natural decantation was observed in the MCh strain, which facilitated operation efforts and minimized centrifugation energy cost.

Temperature and irradiance were typical of the Atacama Desert coastal ecosystem early summer. Temperature values ranged from 25 to 27 °C with 2,385 to 2,599 $\mu E \cdot m^{-2} \cdot s^{-1}$ irradiance. DO conditions in the control and treatment groups remained stable between 9.8 and 11.2 $mg \cdot L^{-1}$ throughout the experiment (Figure 3(a)).

Photosynthetic efficiency (Fv/Fm) is widely used as a stress indicator for culture conditions. Values <0.5 indicate physiological stress in a microalgae culture (White *et al.* 2011). MCh showed no differences in control conditions throughout the experiment, with values around 0.65, describing a 'healthy' photosynthetic performance (Baker 2008). However, during PTWW phycoremediation in the 800-L RW system, MCh showed Fv/Fm <0.5, which progressively increased to statistically the same values as the control (Figure 3(b)). This evidence suggests good adaptability to physiological stress in a short period. In general, microalgae treatments of raw sewage require acclimation to the stress produced by variable nutrient levels and a higher concentration of organics from wastewater (Gupta *et al.* 2016). Previous reports show *C. sorokiniana* and *S. obliquus* with increased stress levels when grown in raw wastewater (Fv/Fm of 0.48) during 15 days. Dilution levels of wastewater provided more favourable conditions for biomass increase and lipid accumulation (Gupta *et al.* 2016). Taken together, results highlight the importance of selecting microalgae strains adaptable to local PWWT harsh conditions in order to scale up the technology to industrial size.

MCh reduced the nitrogen concentration from 36.1 $mg \cdot L^{-1}$ to 5 $mg \cdot L^{-1}$, removing 84% from PTWW (Figure 4(a)). In contrast, phosphorus decreased by 93%

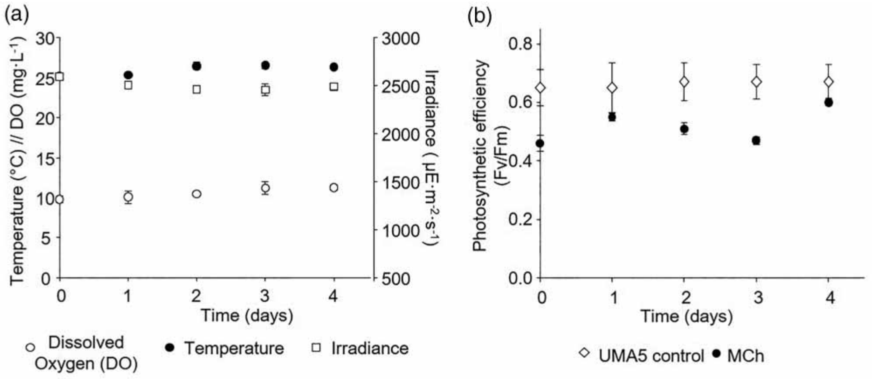

Figure 3 | (a) Environmental factors in 800-L PTWW phycoremediation systems for 4 days. (b) *Muriellopsis* sp. (MCh) photosynthetic efficiency during 4 days of phycoremediation. Different symbols represent UMA5 control and MCh PTWW treatment. Error bars denote differences among treatments after the ANOVA test ($p < 0.05$).

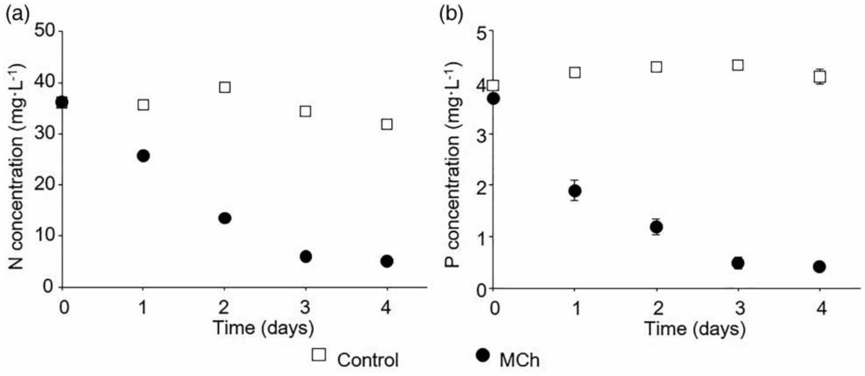

Figure 4 | Nutrient removal in 800-L PTWW phycoremediation systems for 4 days. (a) Nitrogen concentration (N-NH_4 + N-NO_3). (b) Phosphorus concentration (P-PO4). Symbols represent the control and treatment group: see Figure 1 for abbreviation details. Error bars denote differences among treatments after ANOVA test ($p < 0.05$).

(3.7 mg·L^{-1} to 0.3 mg·L^{-1}). Additionally, NBL control conditions remained unchanged (Figure 4(b)). Nitrogen consumption had no statistical differences to MCh in 20-L reactors, suggesting an efficient phycoremediation process at pilot-scale as well.

As for biomass, MCh showed significantly higher growth in PTWW than in the UMA5 culture medium, reaching 760 mg·L^{-1} at the end of the experiment and productivity of 104.3 mg·L^{-1}·day^{-1} (Figure 5(a) and 5(b)). Other studies have reported an increase in *Muriellopsis* sp. biomass during culture in secondary treatment wastewater as well, reaching 500 mg·L^{-1}·day^{-1} productivity under 25-L laboratory-scale conditions without extra nutrients (Gómez *et al.* 2013) Furthermore, *Scenedesmus* sp. grown in 300-L Brite boxes using secondary treatment wastewater showed productivity values (130 mg·L^{-1}·day^{-1}) similar to our results (McGinn *et al.* 2012).

MCh biomass contained 51% protein, 36% carbohydrate and 14% lipid content when cultured in UMA5 control and PTWW. Additionally, carotenoid (0.6%) and lutein (0.3%) contents were similar in both conditions (Figure 6). It seems that stress could redirect the metabolism to increase the accumulation of lipids, carbohydrates and protein (González *et al.* 1997); nevertheless, results show no differences between MCh grown in specific culture medium and MCh during remediation treatment. This evidence shows that PTWW can sustain microalgae growth as efficiently as a specific culture medium, suggesting an effective water treatment alternative which also produces valuable metabolites. Furthermore, it has been reported that high nitrogen concentration increases lutein in *Muriellopsis* sp., and thus continuous protein synthesis supports a massive accumulation of this carotenoid (Del Campo *et al.* 2000). Our results show an increase in carotene accumulation in MCh during phycoremediation, representing a potential as a pigment source for aquaculture, poultry farming, as well as medical applications (Spinola & Santos 2020). However, further

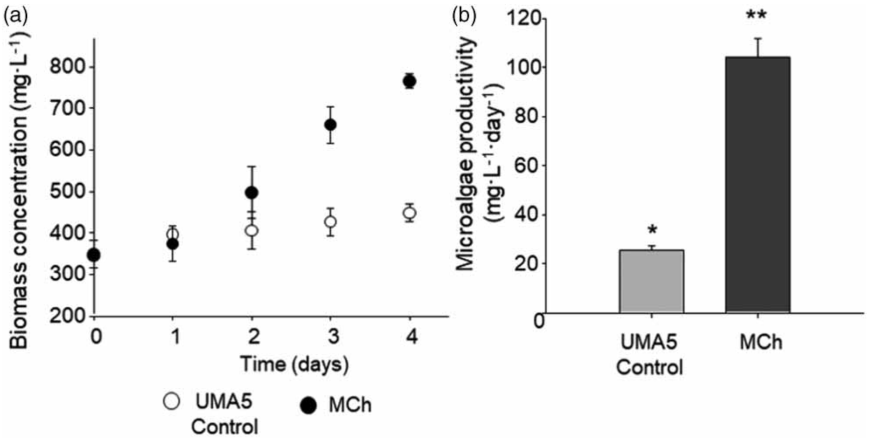

Figure 5 | Performance of microalgae in 800-L PTWW phycoremediation systems for 4 days. (a) MCh biomass concentration. Error bars denote differences among treatments after ANOVA test ($p < 0.05$). (b) MCh productivity. Asterisks denote significant differences after Student's t-test ($p < 0.05$).

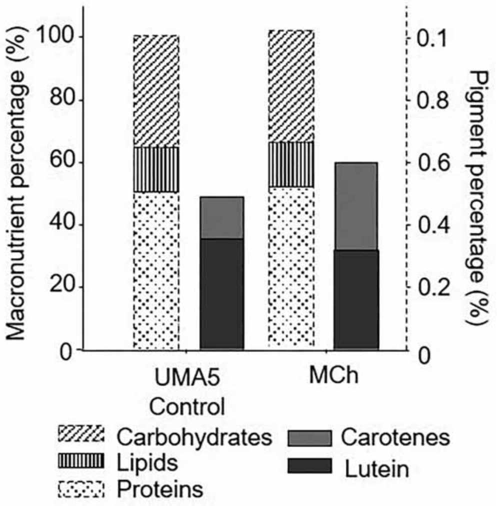

Figure 6 | Biochemical profile of microalgal biomass in 800-L phycoremediation systems after 4 days. Dashed bars represent macronutrient percentage, while solid bars represent pigment content.

research is needed to address toxic compounds accumulation and product safety concerns.

Regarding analysis for irrigation water quality, MCh treatment showed values over 80% lower compared to control values in the maximum quantities of barium, beryllium, cobalt, copper, chromium, fluoride, nickel, lead and vanadium, conductivity and faecal coliform, compared to the NBL control (Figure 7). Furthermore, microalgae-treated water met all Chilean standards for irrigation water quality, except for boron, critic lithium, percentage sodium and chloride. Nonetheless, these parameters do not meet standards after primary treatment in local conventional sewage plants (Supplementary Table S1). Compared to international guidelines and regulations for water reuse (Shoushtarian and Negahban-Azar, 2020), these results do not meet Food and Agriculture Organization (FAO) wastewater guidelines for agricultural use standards and differ in maximum values permitted for other international regulations (Supplemental Table S4). Martínez *et al.* (2018) had previously reported the potential of *Muriellopsis* sp. potential in bioremediation of heavy metals from acid mine drainage located in the Atacama Desert. Results showed copper, zinc and iron removal as high as 79%, 40.4% and 93.5%, respectively, after 12 h treatment and pH 5. MCh treatment on wastewater showed a reduction in copper and iron; however, zinc values had the same maximum in NBL control and treatment groups during 4 days. In all, this evidence suggests that MCh strain could remove different metal and contaminant elements from wastewater, resulting in water of a better quality than the one treated in the local treatment plant (Supplementary Table S1). Nevertheless, further studies are needed to reduce some of the parameters that still do not meet the regulation. It has been proven that good design in microalgal consortia could allow the assimilation, decomposition or accumulation (Zeng *et al.* 2015) of heavy metals and other contaminants (Agüera *et al.* 2020), facilitating the recovery of specific nutrients (Gonçalves *et al.* 2017) based on the characteristics of each wastewater type (Romero-Villegas *et al.* 2018). Overall, these results suggest that adjustments in the bacteria–microalgae consortium, such as a combination of microalgae with different contaminant adsorption ability, are needed to ensure the reduction of all parameters to optimal levels for irrigation water.

External analyses of faecal coliform concentration showed 99.9% in MCh treatment after 4 days (Supplementary Table S1). Replicate samples analyzed in our facilities also showed a 99.9% reduction, from 215×10^6 MPN·100 mL^{-1} to 1.8 MPN·100 mL^{-1} and to 6×10^6 in NBL control (Figure 8), thus meeting Chilean regulation standards for irrigation water quality (1,000 MPN·100 mL^{-1}). This result shows the effect of the microalgae on the reduction of bacterial pathogens. Such effect has been reported previously for *Desmodesmus* sp. grown in open wastewater ponds for 22 days to produce biodiesel, resulting in over 99% removal of total coliform (Komolafe *et al.* 2014). It seems that several microalgal metabolites have antibacterial, antiviral, antifungal, enzyme inhibiting, immunostimulant, cytotoxic and anti-plasmodial features (Acurio *et al.* 2018); however, further studies are needed to characterize such properties in the MCh strain.

Moreover, bacterial systems exposed to sunlight can be inactivated by a complex set of interacting photo-physical, photo-chemical and photo-biological mechanisms (Nelson *et al.* 2018). Coliform bacteria reduction in NBL control system could be attributed to the RW system itself, which maximizes the efficiency of microalgal cells to perform photosynthesis, and thus maximizing sunlight exposure to all particles within the system. Overall, our results show that MCh treatment in RW systems displaced faecal coliform in local environmental conditions, suggesting a synergic effect of sunlight, microalgal biomass, microalgae metabolites and DO finally contributes to water disinfection.

CONCLUSIONS

The application of microalgae to remediate wastewater, recycle and increase water availability in the Atacama Desert coastal region has real potential, and its applicability

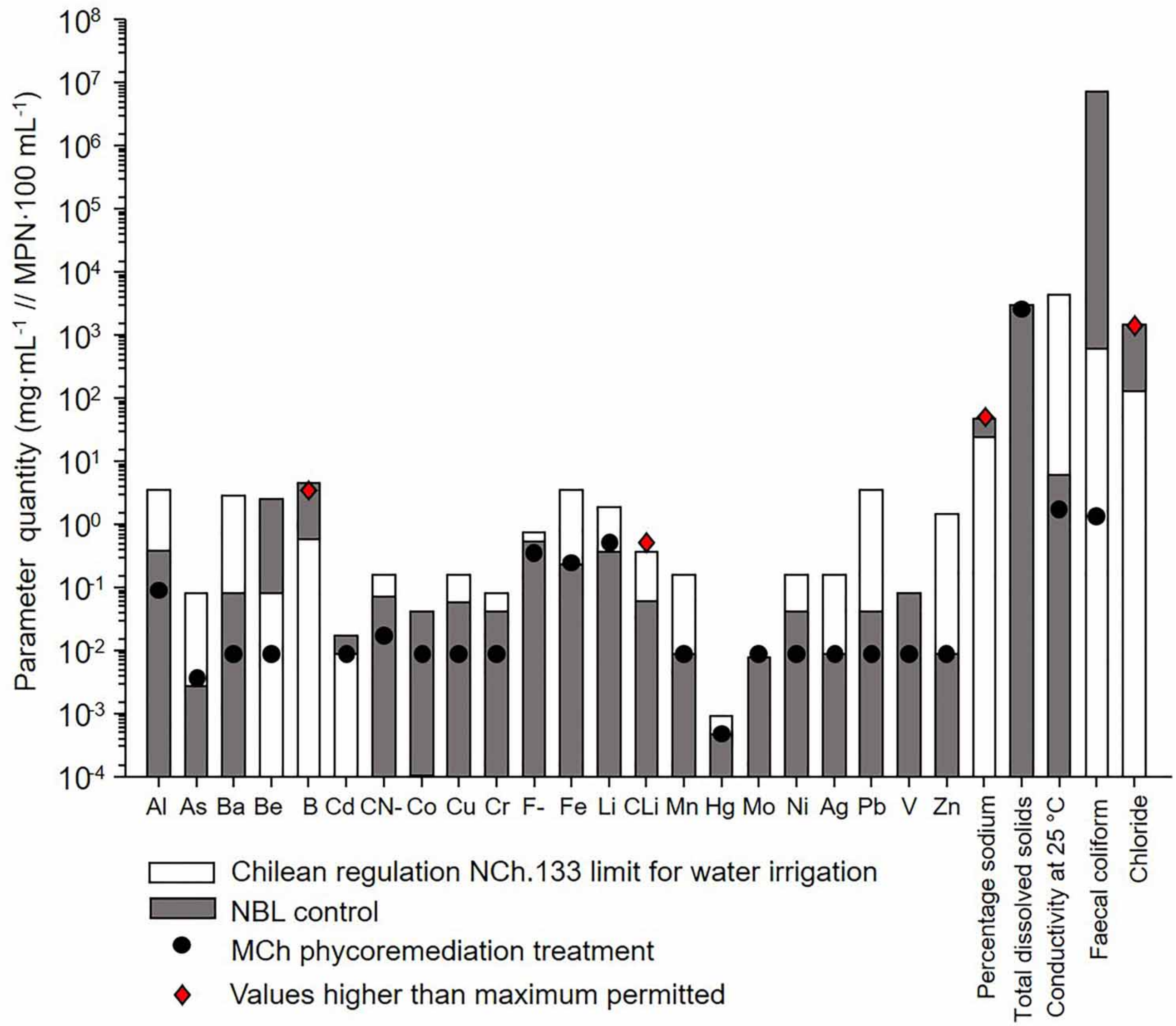

Figure 7 | Water quality analysis performed by Environmental Laboratory SGS Chile Ltd in NBL control and PTWW, after 4 days. Dark grey bars represent maximum concentration in NBL control, whereas bold bars represent Chilean regulation (NCh. 1333) maximum limit allowed in irrigation water (mg·mL^{-1} for chemical elements and MPN·100 mL^{-1} for faecal coliforms). Black circles show maximum values in MCh treatment. Diamonds show MCh parameters that did not meet the standard.

could be scaled to other locations. In the present study, *Muriellopsis* sp. was the most efficient microalgae in nutrient removal throughout volume up-scaling treatments. Phycoremediation of PTWW using MCh treatment can enhance the water quality by reducing nitrogen and phosphorus concentration as well as the faecal coliform load and several contaminant elements. Furthermore, wastewater treated with MCh meets most of the parameters of Chilean regulation for irrigation water; however, the reduction of toxic ions concentration needs to be further studied in order to agree with FAO and other international guidelines and regulations, by improving microalgae consortia or recirculating PTWW. Additionally, MCh was able to increase biomass growth throughout treatments, proving that nutrient recycling from PTWW was possible. Biomass biochemical composition had no difference whether the microalgae were grown in PTWW or specific culture medium. They both represent a good source of proteins and carotene pigments that could be used for animal feed and other applications; nevertheless, further research is needed to address product safety concerns.

The work considered in this study has helped to assess an alternate method for conventional water treatment plants, which adds value to each production step, recovering

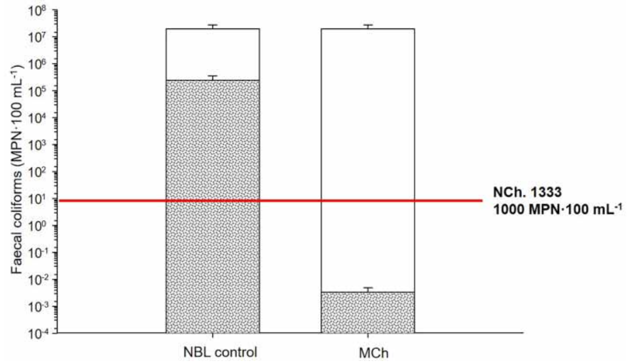

Figure 8 | Faecal coliform bacteria: MPN·100 mL in 800-L phycoremediation systems after 4 days of treatment. Bold bars represent concentration on day 0, while dotted bars, at day 4. Horizontal line indicates faecal coliform value permitted in irrigation water by Chilean regulation. Significant differences were found within both groups after Student's t-test ($p < 0.05$).

water with irrigation quality and biomass with high potential for different usage. This biotechnological application represents a promising circular economy-based process for wastewater reclamation in the Atacama Desert. Future research could address the characterization of microalgae consortium efficiency on nutrient and contaminant removal under local conditions and final product safety, in order to identify the feasibility at industrial scale and prolonged treatments.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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