

Algal biomass production in different types of wastewaters under extreme conditions of light and temperature

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ABSTRACT

Microalgae have been considered as a potential feedstock for biomaterial compounds production. In the southern Mediterranean region, which suffers from water scarcity, using wastewaters as culture media for microalgae cultivation is recommended. Hence, an algal strain is needed, which exhibits high growth under the prevailing temperature and light conditions. To obtain such strains, natural samples were collected from a maturation pond in the Peri-Urban Area of Marrakech (Morocco) and cultivated in different types of wastewaters at light intensity of $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and temperature up to 45°C in a batch system. Three types of wastewaters were used: raw, treated, not sterilized treated domestic wastewater from multi-soil layering system and tap water, started with the same concentrations of nitrogen and phosphorus. Results showed *Chlorella* was the only strain able to grow under the cultivation conditions established. This microalgae was identified by partial *rbcL*-gene as *Chlorella sorokiniana* with a genetic similarity of 98.8%, and registered under UCAM 001 (GenBank number MT999855). This finding indicates that the strain can be used for biomass production and phycoremediation process in semi-arid area. In addition, microalgae isolation by dominance could be a fast approach to obtain adapted strains for outdoor mass cultivation.

Keywords: Microalgae; Isolation by dominance; Wastewaters; Extreme conditions; Biomass production

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1. Introduction

Optimization of microalgae production is receiving a growing attention due to their potential to produce many valuable products (drugs, vitamins and energy, etc) [1]. For the industrial production of bioenergy and biomaterial products from microalgae, it is crucial to evaluate the feasibility of scaled-up outdoor culture systems. Some culturing systems were reported for massive cultivation of microalgae under outdoor conditions, such as: raceways ponds, tubular, column and flat plate photobioreactors [2]. The conventional systems as open ponds face some limitations like a vast occupied area requirement, contamination, non-sufficient level of control over the process, deficient penetration of light, and considerable evaporation rate [3]. Photobioreactors (PBRs) provide a better process control and optimization due to their design (higher productivity, capability for the cultivation of sensitive microalgae species, efficient light accessibility through the culture) [4]. However, microalgae which reach high growth rates in wastewater are not those presently used for biomass production in laboratory [5,6]. For outdoor cultivation with varying conditions in light intensity, light-dark cycles, and temperature, the selection of microalgae strains adapted to the specific environmental conditions (light and temperature) prevailing in different areas is needed [7–10].

Among the challenging problems encountered during industrial biomass production are the high cost of synthetic nutrients added and water availability [11,12]. In order to reduce costs, the use of wastewater as a medium for microalgae cultivation is highly recommended [13–15], especially in arid and semi-arid areas that suffer from water scarcity. Integrating wastewater and algal biomass production therefore provides cumulative benefits, eliminating the need of external water and nutrients supply [16]. Indeed, according to some authors, microalgae culture

systems can be coupled with wastewater treatment technologies for different kind of valuable products production such as cosmetics, fertilizers, feed, nutraceuticals and pharmaceuticals [17–20].

This study aims to investigate the effects of high temperature and high light conditions on microalgal biomass production grown in different wastewaters enriched with nutrients using a batch pilot (simulating the flat plate photobioreactor conditions located in Marrakech City, a semi-arid area in Morocco). Different light intensities and temperatures were tested in order to study the tolerance of the microalgae to the summer conditions of Marrakech City (south Morocco). To obtain such a microalgae strain well adapted to the extreme conditions (high temperature and light) of the location, the approach of selection and isolation from natural water by dominance was applied. This new approach was conducted in order to favor those microalgae strains, which are able to resist and be adapted to these extreme conditions. Hence, incubation and cultivation were going on until a species become dominant which could thus be easily isolated. The obtained isolates could be selected and can be qualified as a strain tolerant to the PBRs and to light-temperature summer conditions. Thus, it will be preferably used for outdoor mass cultivation in the southern Mediterranean regions.

2. Material and methods

An overview of all experiments conducted in this study is shown in Fig. 1.

2.1. Microalgae cultivation

The microalgae mixture was collected from the wastewater treatment plant Saada Lagoon (WWTP) located at 18 km west of Marrakech City Center, Morocco

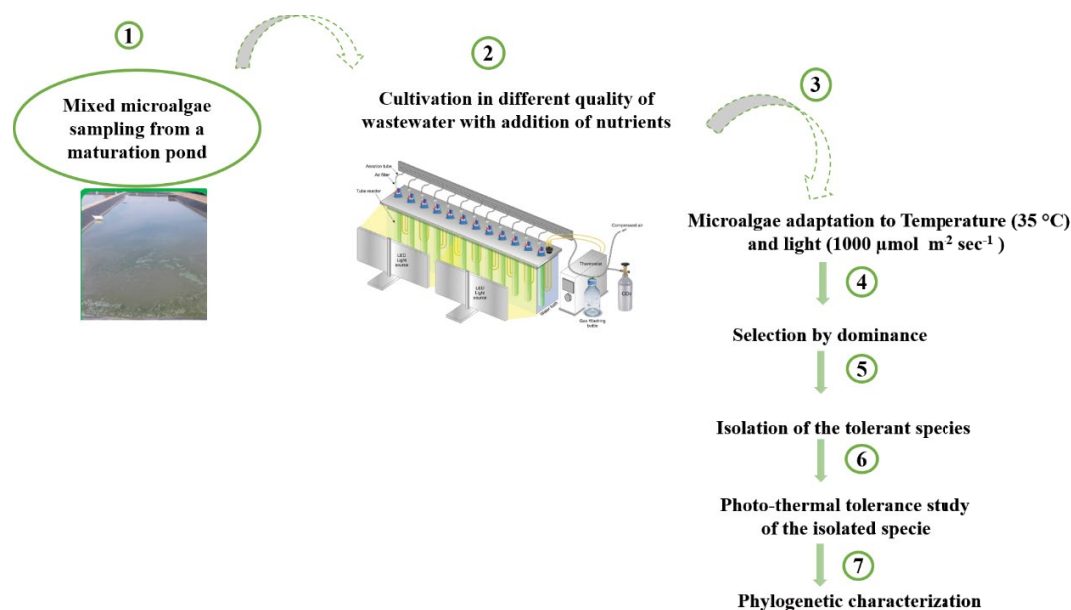


Fig. 1. Overview of the experiments conducted in the present study, sampling and different cultivation conditions in the batch system.

(31°39'16"N and 8°06'59"W) in January 2020, reflecting winter conditions. The wastewater treatment facility consists of 1 anaerobic pond (primary treatment), in series with 1 facultative pond (secondary treatment), in series with 1 maturation pond (tertiary treatment) treating domestic wastewater from Peri-Urban Area. Microalgae grow randomly in the facultative and maturation ponds under non controlled conditions, where sampling was done. These ponds are characterized by high density of Chlorophyceae and Euglenophyceae (Fig. 2).

The physico-chemical characteristics of the water samples are presented in Table 1.

Three types of wastewaters were tested: raw wastewater (RWW) filtered with membrane filter MF-Millipore (0.45 µm), treated wastewater (TWW) and treated sterilized wastewater (TSWW). Raw and treated domestic wastewater were collected from the inflow and outflow of a hybrid multi-soil-layering (MSL) plant located at the faculty of law, Marrakech, Morocco (31°39'16"N; 8°06'59"W). Table 2 summarizes the physico-chemical characteristics of the different media. Tap water was used in order to detect if there are any other elements, in the other media, besides nitrate (NO₃⁻) and orthophosphates (PO₄³⁻) that could enhance the growth of microalgae.

To all types of waters, nitrate and orthophosphate were added to reach a total concentration of 100 mg N L⁻¹ and 10 mg P L⁻¹, respectively, in order to avoid mineral growth limitation [21,22].

The high concentration of nitrate (NO₃⁻) in TWW is explained by the process of the hybrid MSL which is based on nitrification.

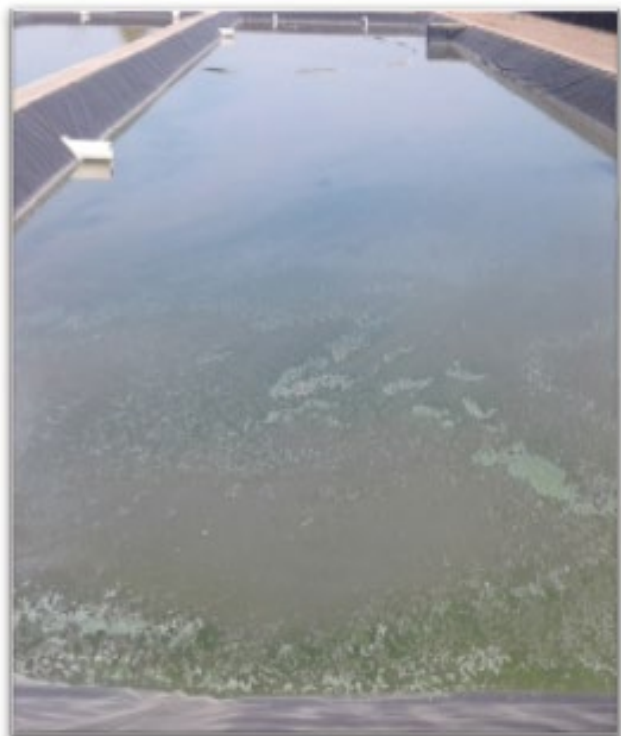


Fig. 2. Saada Lagoon Maturation Pond (Peri-Urban Area of Marrakech City).

2.2. Physico-chemical analyzes

Physico-chemical parameters, COD (chemical oxygen demand), TN (total nitrogen), NH₄⁺ (ammonium), NO₃⁻ (nitrates), TP (total phosphorus), PO₄³⁻ (orthophosphates) were characterized by standard methods AFNOR and Rodier et al. [23,24]. The water temperature, electrical conductivity (EC) and pH were determined in situ with a multimeter instrument (Orion 4 Star).

2.3. Batch culture experiment

The batch cultivation was done in glass tubes of 490 H × 40 D (mm) holding a volume of 350 mL. 12 glass tubes were submerged into a transparent acrylic glass water bath and kept in a vertical position in black acrylic brackets. These brackets prevented ambient light from reaching the tubes from the back and the sides.

300 mL of the culture media were added (RWW, TWW, TSWW and tap water) in each tube, each in triplicate. N and P concentrations were first measured in the waters used for culture and subsequently nitrate and orthophosphate were added to obtain final concentrations of 100 mg N L⁻¹ and 10 mg P L⁻¹, respectively. As an inoculum the microalgae mixture sampled from the maturation pond was added at amounts to each tube to reach an optical density of 0.4 at 680 nm. To simulate the conditions prevailing in the southern Mediterranean the temperature was kept constant at 35°C and the photosynthetic active radiation (460–700 nm) provided by LED lamps at 1,000 µmol m⁻² s⁻¹ (Esbaybulbs®, Product No. HZW0179283DE). All tubes were irradiated from the front side of the water bath. The cultivation was done in a batch system in which turbulent mixing was established with a constant flow of air. Temperature was kept constant with a thermostat (Fig. 3). Samples were taken daily and species abundances were determined by cell counts using the Malassez Hemocytometer (depth 0.2 mm) comprising a grid engraved on the glass and delimiting a surface of 5 mm². The grid was filled with the biological sample diluted with distilled water. Each rectangle of the plate had a surface area of 0.05 mm² corresponding to a volume of 10⁻⁵ mL. The cells concentration *N* was calculated by:

$$N = \frac{n}{V} \times f \quad (1)$$

Table 1
Physico-chemical characteristics of Saada Lagoon Maturation Pond used for sampling microalgae in January (mean ± standard deviation)

pH	8.25 ± 0.01
TSS, mg L ⁻¹	140 ± 18
COD, mg L ⁻¹	153.7 ± 3.6
NH ₄ ⁺ , mg L ⁻¹	43.29 ± 2.2
NO ₃ ⁻ , mg L ⁻¹	4.25 ± 0.34
TP, mg L ⁻¹	6.11 ± 0.31
PO ₄ ³⁻ , mg L ⁻¹	3.43 ± 0.2
<i>T</i> , °C	12 ± 0.5

TSS: Total suspended solids.

Table 2
Physico-chemical characteristics of different media used for cultivation of microalgae

Parameters	RWW	TWW	Tap water
pH	8.22 ± 0.03	8.04 ± 0.03	7.22 ± 0.03
Conductivity ($\mu\text{s cm}^{-1}$)	1172 ± 0.02	715.99 ± 0.02	1002.5 ± 3.47
TN (mg L^{-1})	38.33 ± 0.28	7.83 ± 0.57	–
NH ₄ ⁺ (mg L^{-1})	32.69 ± 0.64	6.11 ± 0.07	0.02 ± 0.03
NO ₃ ⁻ (mg L^{-1})	0.35 ± 0.02	6.90 ± 0.03	5.99 ± 0.53
TP (mg L^{-1})	4.41 ± 0.23	1.45 ± 0.04	–
PO ₄ ³⁻ (mg L^{-1})	3.10 ± 0.02	1.28 ± 0.01	0.01 ± 0.01

RWW: raw wastewater; TWW: treated wastewater; Tap water.

where the n is the number of cells counted, V the volume of counting tiles given as 10^{-5} mL, f the dilution factor and N the number of cells per mL.

In addition, cell density was measured by optical density at 680 nm (OD_{680}) [25] using a spectrophotometer. The experiments were finished when the species that amounted to 80%, reached the decline phase of the algal cells present in the culture medium and thus became dominant.

2.4. Temperature tolerance

Prior to the experiments, single microalgae strains were cultured in a benchtop system described above to reach a biomass concentration of 1 g L^{-1} at a temperature of 25°C , a pH of 7 ± 0.5 , constant purging with air containing 5 vol.% of CO_2 , and irradiation with PAR of

$1,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. During the experiment, the temperature was increased in 5°C steps from 35°C to 50°C based on a previous study [26]. This range of temperature was chosen in order to simulate temperature summer conditions prevailing in Marrakech City. To allow the microalgae to adapt to the temperature, each temperature level was kept constant for 1 h. After that period a sample of about 1 mL was taken and analysed by pulse amplitude modulation (PAM) fluorometry using a Dual-PAM-100 (Waltz, Germany). Thereby, the maximum quantum yield (F_v/F_m) of photosystem II (PSII) was determined upon 5 min of dark adaptation time. The maximum saturation pulse was applied for 300 ms and a photon flux density of $20,000 \mu\text{mol m}^{-2} \text{ s}^{-1}$. In addition to the *Chlorella* strain isolated at the end of the experiment from Saada Lagoon, *Chlorella sorokiniana* strain

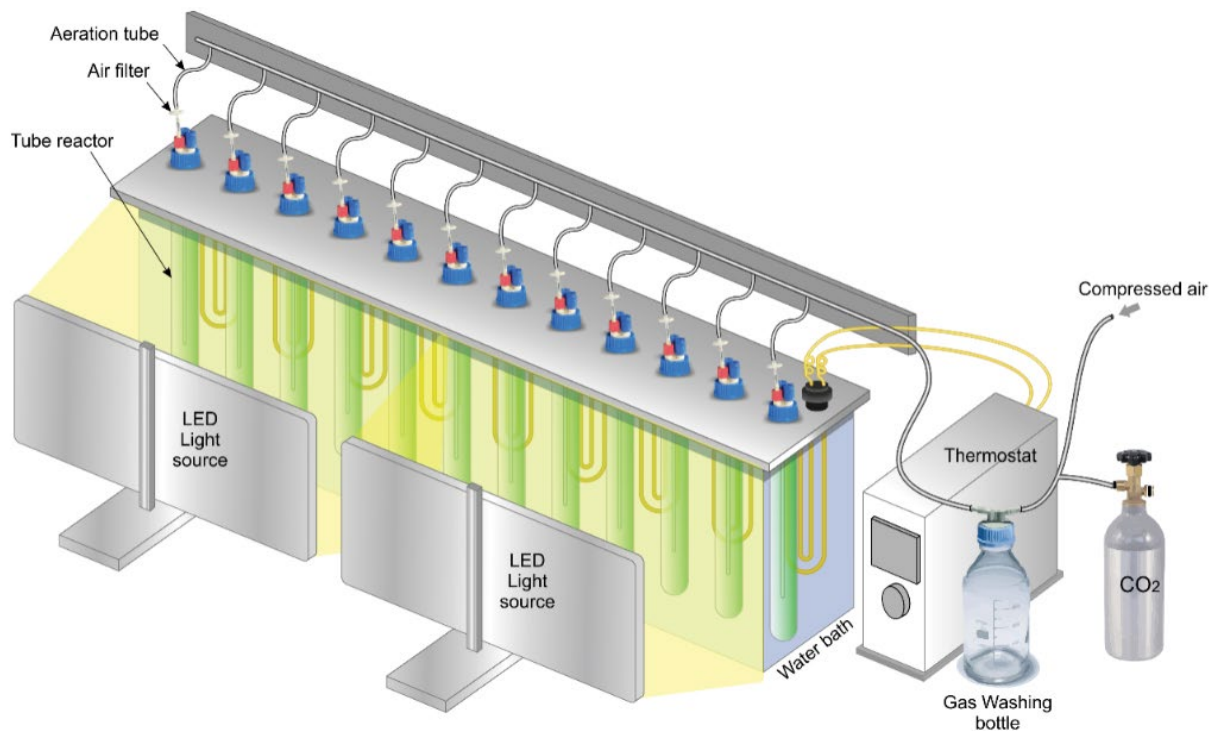


Fig. 3. Scheme of the benchtop batch system for algal cultivation.

SAG 211-8K and *Chlorella vulgaris* strain U 213 (1026) from the collection of University of Hamburg were analysed for comparison on temperature tolerance.

2.5. Biomass production

Biomass production of the *Chlorella sorokiniana* strain sampled and isolated from Saada Lagoon were determined during batch cultivation for 72 h in the benchtop system described above (Fig. 2). As references *Chlorella sorokiniana* strain SAG 211-8K was studied. Cultivation was done at a constant temperature of 30°C and continuous purging (2 L/min/tube) with air enriched in CO₂ to 5 vol.% [27,28] and a photosynthetic active radiation of 700 and 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. All experiments were done in triplicates. Potassium nitrate and Flory 2 (Planta Düngemittel GmbH, Germany) were added to reach a starting concentration of N and P of 440 and 120 mg L⁻¹, respectively, which was shown in previous experiments to allow constant growth up to a biomass concentration of 5 g L⁻¹. Samples were taken from the cultivation tubes once a day around midday and immediately analysed for optical density at 680 nm. Dry weight was calculated from

OD 680 using a conversion factor of 0.162, determined previously.

2.6. Phylogenetic analysis of the microalgae

Total DNA was extracted for the phylogenetic analysis using the NucleoBond high molecular weight genomic DNA kit for microorganisms (Macherey-Nagel, Germany) following the manufacturer's instructions and a previously published enzymatic cell lysis protocol with some modifications, including freezing in liquid nitrogen, bead beating, and an additional lysis pretreatment with proteinase K and lysozyme for 24 h at 55°C [29,30]. For the phylogenetic characterization of the new isolate gene-regions of the ribulose biphosphate carboxylase (*rbcL*) were amplified using oligo-nucleotide primers with an additional sequencing adapter-binding site (M28F and M1390R: 5'-GGT GTT GGA TTT AAA GCT GGT TGT-3' and 5'-CTT TCA AAY TTC ACA AGCAGC AG-3' [31]. PCR mixtures contained 100 ng of template DNA μL^{-1} , 0.2 mM of each of the four deoxynucleoside triphosphates, 1.5 mM MgCl₂, 1 μM (each) primer, and 2.5 U of Taq DNA polymerase. Thermocycling conditions included 45 s of denaturation

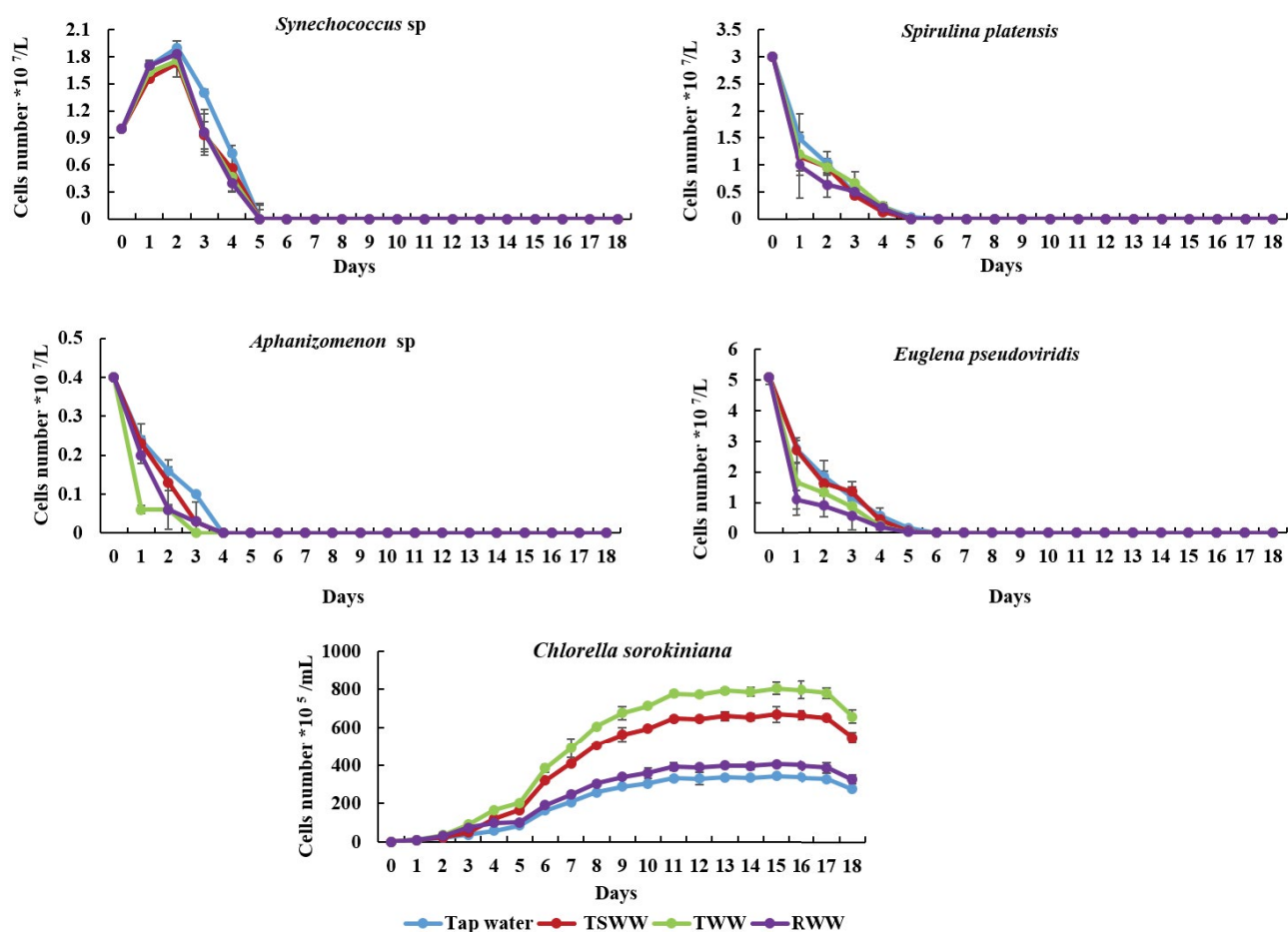


Fig. 4. Evolution of the cell numbers of the microalgae present in the natural sample from the maturation pond at a temperature of 35°C and a PAR radiation of 1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in different culture media.

at 95°C, 45 s of primer annealing at 56°C, and 2 min of primer extension at 72°C. This cycle was repeated 34 times. The fragment was sequenced with automated ABI377 technology following the manufacturer's instructions.

3. Results and discussion

3.1. Isolation of microalgae by dominance

At the beginning of the experiment, the microalgae mixture consisted of the species *Euglena viridis*, *Spirulina platensis*, *Aphanizomenon* sp., *Synechococcus* sp. and *Chlorella* sp. All these microalgae were cultivated, in the culture media tested, all together for the screening.

Except, of *Euglena viridis*, *Spirulina platensis* and, *Aphanizomenon* sp., all microalgae present in the natural samples exhibited growth in the wastewater media (Fig. 3). Tap water was even less attractive than wastewater to most of the microalgae in the mixture and only *Synechococcus* sp. showed any growth in it. Marked differences in growth were also detected in the sterilized and the non-sterilized media indicating that most of the microalgae needed bacteria for growth. This finding was already described in literature and is discussed in more detail below. In summary results indicate that wastewater used for culture medium was not inhibited growth to any of the microalgae species present in the natural sample. This could be expected and was intended by sampling the microalgae from a maturation pond which reflects wastewater conditions.

The finding that algal growth always started after a lag phase of between two to three days indicates that all microalgae under study had to adapt to the high radiation and temperature established during the experiments [32]. To what extend high radiation or high temperature were ruling growth cannot be decided from the cell counts, but only from literature which is discussed below.

For the cyanobacteria *Spirulina platensis* the ability to grow in wastewater at temperatures between 25°C and 35°C was shown [33,34]. Thus, the temperature was not the ruling factor that prevented *Spirulina* from any growth in our experiment. However, *Spirulina* is sensitive to pH and prefers high alkaline and saline media [35,36]. A pH of up to 9 was reached only at the beginning of the experiment. The absent growth of *Spirulina* sp. was probably due to the high radiation as it could be also explained by bacteria competition for nutrients with microalgae that could reduce the level of their availability [10]. Also, a light-duty-cycle efficiency reduction factor could be a critical parameter for *Spirulina* non growth in these wastewaters due to the presence of solids and other particles in the wastewater that could interfere in the distribution of light [37].

The *Aphanizomenon* sp. decrease from winter results has been already demonstrated in previous studies and explained by the preference of this specie to low temperature. The strain could grow at above 8°C with an optimum temperature ranging from 23°C to 29°C under low light conditions [38,39].

Present results showed that *Synechococcus* sp. grew rapidly until day 2 dominated the species composition before

decreasing subsequently (Fig. 4). The optimal growth conditions of this strain was found in literature to be 38°C (able to tolerate up 43°C) observed at a light intensity of 660 $\mu\text{mol m}^{-2} \text{s}^{-1}$ [40]. This indicates that present temperature used is not a ruling factor to *Synechococcus* sp. growth. However, their growth was sensible to extreme irradiance combined with extreme temperature.

As shown in Fig. 4, *Euglena pseudoviridis* density decreased significantly from the first day of the experiment till the end. This species could not tolerate high temperature (35°C), light (1,000 $\mu\text{mol m}^{-2} \text{s}^{-1}$) and bubbling. This result was widely proved by several studies which reported that *Euglena* sp. has special requirements of temperature and light for its growth. Indeed, the extreme light and temperature intensity affects the photosynthesis process that could lead to less biomass [41,42]. Other authors reported that the ideal temperature for the multiplication of *Euglena* cells is 25°C–30°C [43]. In addition, many studies have proved that the bubbling causes flagellate algae stress [44,45]

The present species was thus clearly not able to tolerate the high temperature (35°C) established during the experiments and thus showed no growth at all.

The present results showed that the lag phase of *Chlorella* sp. was longer than of the other microalgae species indicating that high temperature and high radiation produced a significant stress on this species [46]. However, after 3 d a marked and constant increase in cell numbers was observed in TWW and TSWW indicating efficient adaptation to both high temperature and high radiation (Fig. 4). That dominance was reached after 5 d indicates that the growth rate is sufficiently high to out-compete other species. The finding that *Chlorella* did not grow well in sterilized wastewater as it did in TWW indicates that bacteria could be essential for growth. This is in accordance with some authors who showed a decreased growth rate and lipid content in *Chlorella* density when isolated from their full microbial community [47]. This is explained by that bacteria use oxygen produced by photosynthesis for organic carbon degradation and release nutrients and inorganic carbon, which are substrates for microalgae and autotrophic bacteria [44,48,49]. In addition, the bacteria provide growth-promoting metabolites [45], such as vitamin B12 [50,51] and heterotrophic bacteria can reduce oxidative stress experienced by microalgae [52]. The finding that none sterilized media is well to produce high biomass of microalgae provides a new perspective for the screening and the identification of the positive bacteria contained in MSL treated wastewater and their possible isolation to enhance or simulate the growth of the microalgae for future large-scale cultivation.

3.2. Nutrient removal

The phosphorus concentration (P) in all the media tested, decreased during the first 4 d of cultivation (Fig. 5); a significant removal rates were shown between all the media tested in this study ($p < 0.05$). The highest efficiency in P removal was achieved from the culture of TWW and RWW where the removal rates reached 74.9% and 72.45% at the end of the 4th day, respectively. By the end of the experiment, it reached 100% for TWW and TSWW

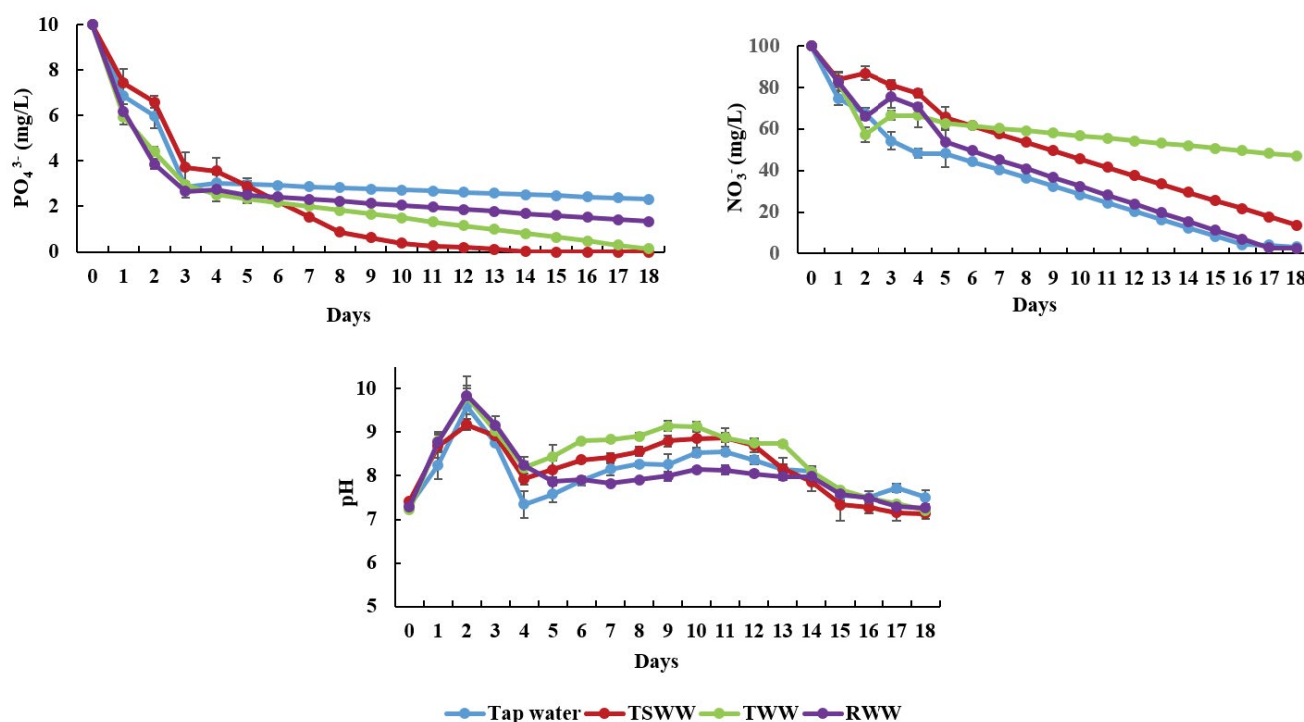


Fig. 5. Evolution of PO_4^{3-} , NO_3^- and pH during the cultivation of the microalgal species of Saada Maturation Pond in the tested media (Tap water; TSWW: treated sterilized wastewater; TWW: treated wastewater; RWW: raw wastewater) at a temperature of 35°C and a PAR radiation of $1,000 \mu\text{mol m}^{-2} \text{s}^{-1}$.

The removal rates mentioned above of phosphorus decreases rapidly within 4 d. These removal efficiencies were higher and faster than the values obtained for mixotrophic algal consortia in municipal wastewater reported by Mahapatra et al. [53] (removal efficiency was 78% (22.2 ± 2.4 to $5.13 \pm 1.67 \text{ mg L}^{-1}$) occurred in 14 d).

The fast decrease of P concentration during the first 4 d of the experiment can be explained by luxury uptake which is known to increase with temperature [54,55]. At high temperature the removal of phosphorus co-precipitate with metallic ions in alkaline conditions which is an important process for phosphorus removal. Van Den Hende et al. [56] reported abundant mineral formation (such as hydroxyapatite and struvite) in microalgae culture using wastewater due to the increase of the solution pH. The formation of these minerals is a relatively slow process. However, although in all batch experiments luxury uptake produced a decrease of P to below 2 mg P L^{-1} after 3 d it can be assumed that microalgae growth was not limited by P until the end of the experiments. The phosphorus was incorporated at amounts of around 8 mg P L^{-1} which according to Redfield Ratio allows a production of biomass of around $1.6 \text{ g dry weight L}^{-1}$.

In addition to the luxury uptake, bacteria have also the ability to degrade the organic phosphorus [57], what explains the high removal rate in TWW and RWW. Uba [58] showed that the wastewater is highly rich in microorganisms such as *Pseudomonas* and *Acinetobacter*. The later was identified as the bacterial genus mainly responsible for the reduction of phosphorus [59].

However, it has been noticed a high fluctuation in nitrogen concentrations during the first days of the experiment. The variation in nitrate can be explained by nitrification process described previously for batch cultivation of microalgae when ammonium is present [10,60]. Many authors have shown this phenomenon during batch culture of microalgae [34,33]. It has been shown also that nitrification and denitrification processes depend on many factors such as dissolved oxygen and chemical oxygen demand/nitrogen ratio [61–63]. By the end of the batch culture experiment, the removal of nitrates was more efficient in tap water, RWW and TSWW in comparison to that in TWW.

3.3. Characterisation of the isolated microalgae strain

Phylogenetic characterization of the *Chlorella* strain sampled and isolated during the experiment revealed a similarity to the genus *Chlorella sorokiniana* by 98.8% (Fig. 6). The phylogenetic tree highlighting the position of *Chlorella sorokiniana* relative to the type strains of other species within *Chlorella*. The phylogenetic tree was generated using MEGA X Bootstrapped Neighbour-Joining analysis based on the MSA (multiple sequence alignment) implemented in MEGAX_ClustalW [64–66].

As becomes obvious from Fig. 7 all *Chlorella sorokiniana* strains under study showed the same temperature tolerance and maintained maximum photosynthetic activity up to 45°C , this is in accordance with previous studies on *Chlorella sorokiniana* temperature tolerance. Li et al. [67] and Varshney et al. [68] studied the effect of different temperature on

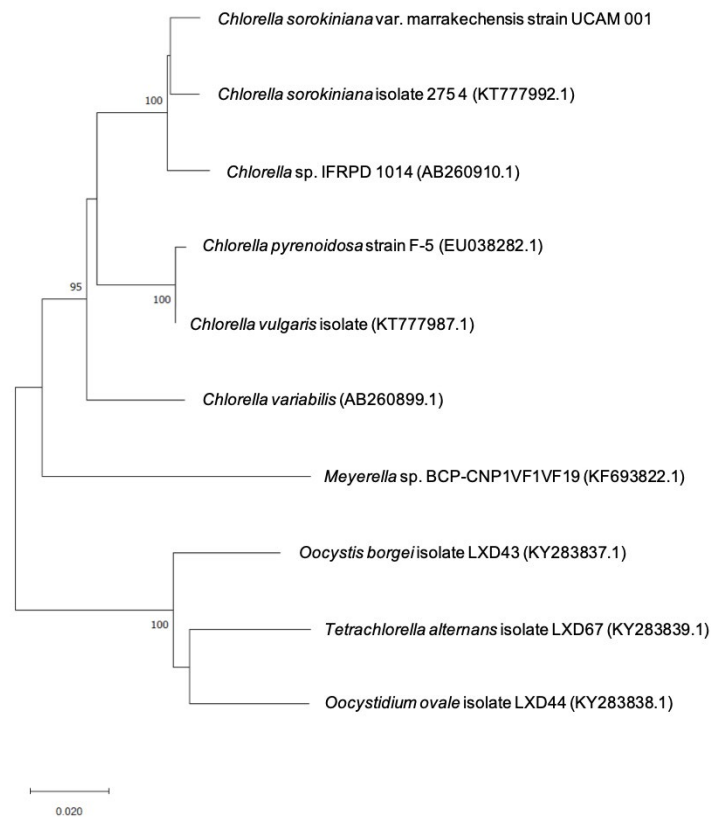


Fig. 6. The phylogenetic tree highlighting the position of *Chlorella sorokiniana* relative to the type strains of other species within *Chlorella*. The phylogenetic tree was generated using MEGA X Bootstrapped Neighbour-Joining analysis based on the MSA (multiple sequence alignment) implemented in MEGAX_ClustalW.

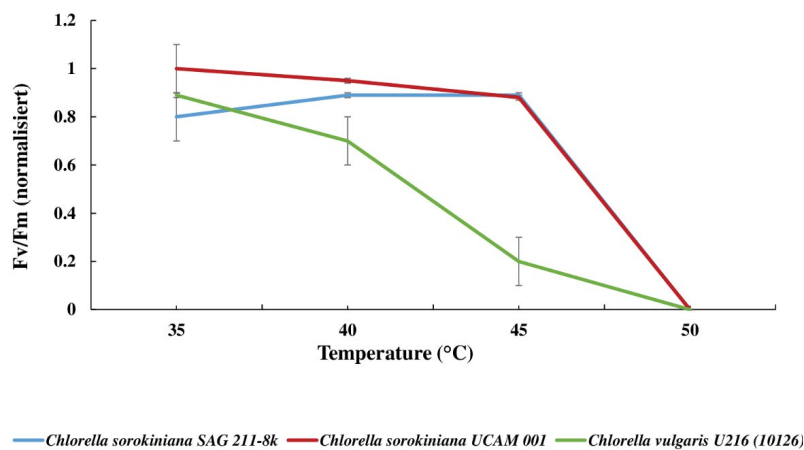


Fig. 7. Comparison of the changes of photosynthetic activity (F_v/F_m) with temperature of *Chlorella sorokiniana* strain SAK 211-8k from Texas and *Chlorella sorokiniana* isolated from Saada Maturation Pond as well as a *Chlorella vulgaris* strain obtained from the collection at University of Hamburg.

Chlorella sorokiniana growth, and showed a temperature tolerance up to 43°C. In contrast, *Chlorella vulgaris* which is widely used for mass cultivation decreased in photosynthetic activity already at 35°C. There are several reports on the influence of the temperature on the productivity and growth rate of *C. vulgaris*. The study by Suthar and Verma [69] had revealed

that *C. vulgaris* growth was decreased when temperature rise above 30°C, which was in concur with the result of present finding. Another study done by Ievina and Romagnoli [70] who had observed the non-resistance of the strain to high temperature, already at 30°C considerable decrease in cell viability, optimal temperature of the species is between 25°C

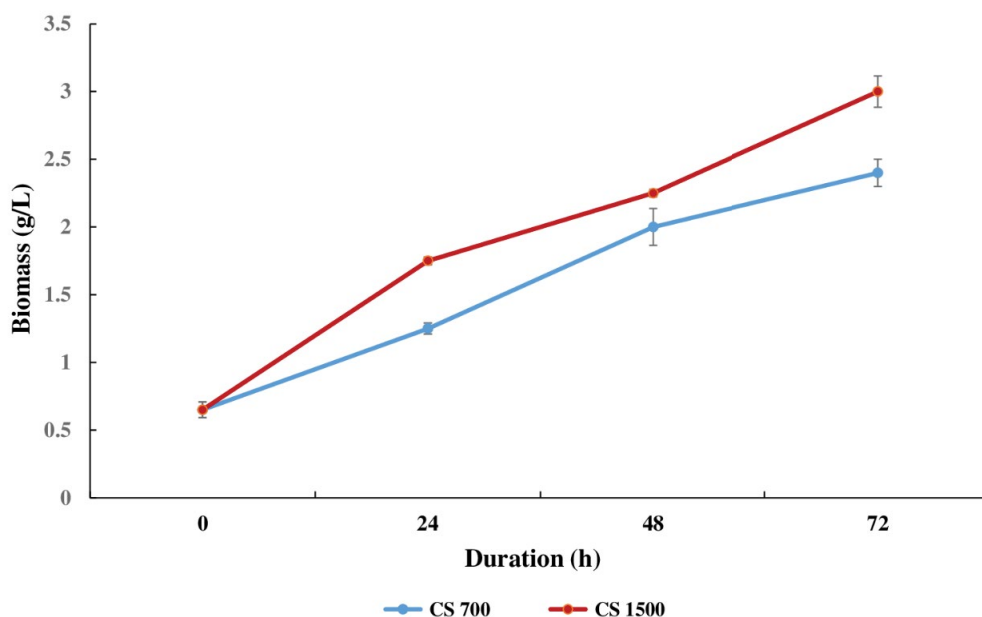


Fig. 8. Increase of biomass during cultivation of *Chlorella sorokiniana* isolated from Saada Lagoon for 72 h at 30°C and a PAR of 700 and 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ respectively.

and 28°C. The tolerance of high temperatures makes our *Chlorella sorokiniana* strains clearly favorable for mass cultivation in the southern Mediterranean where high ambient temperatures and radiation prevail.

As becomes obvious from Fig. 8 the *Chlorella sorokiniana* strain isolated from Saada Lagoon (Morocco) showed significant marked differences in growth ($p < 0.05$) when exposed to high and medium PAR intensities. While at 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ the *Chlorella sorokiniana* grew faster, the reverse was observed at a radiation of 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Many authors have already reported the effects of increasing light intensity and temperature on a microalgae species' biomass production or photosynthetic oxygen evolution rate and give useful knowledge about potential photo-inhibition, the saturating light intensity and the quantum efficiency [71–74]. According to these authors, extreme conditions of light and temperature did not exhibit good biomass production and photosynthetic activity for most of microalgae species and also *C. sorokiniana* strains tested.

However, some *Chlorophytes* are reported to tolerate up to 55°C, but *C. sorokiniana* strains tolerate up to 42°C [73]. To the best of our knowledge, never the combination between high temperatures (up to 45°C) and light (up to 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$) has been suitable for *C. sorokiniana* strains growth. This new finding makes the strain a formidable candidate for industrial applications under local climate conditions (semi-arid area) that allow the use of the new strain *C. sorokiniana* UCAM 001 for outdoor mass cultivation. Moreover, this strain was able to grow in wastewaters with very high nutrient concentrations (100 and 10 mg L^{-1} of N and P, respectively). Obtained results encourage the use of wastewaters that are rich in

nutrients (RWW and TWW) as culture media to minimize costs in medium preparation in order to produce high biomass and to set up the tertiary treatment of wastewater by phycoremediation at the same time.

4. Conclusion

In the present work, different quality of media was used for the cultivation of a microalgae mixture (*Euglena viridis*, *Spirulina platensis*, *Aphanizomenon* sp., *Synechococcus* sp. and *Chlorella* sp.) sampled from a maturation pond in Morocco. Different light intensities and temperature were studied in order to test the tolerance of these strains. Results showed that a removal efficiency higher than 70% and 30% of phosphorous and nitrates, respectively was achieved with the TWW where *Chlorella* strain grew well. *Chlorella* was the only dominant species under the conditions established and showed a 98.8% similarity with *Chlorella sorokiniana*. This identified new strain *C. sorokiniana* UCAM 001 shown to have a heat tolerance up to 45°C and a good biomass at high radiation of 1,500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The ability of this strain to grow under extreme conditions (high radiation, temperature) similar to those prevailing in semi-arid climate conditions makes it suitable for outdoor cultivation in flat plate photobioreactor, already established in our faculty that could be used in different domains and leading to a cost reduction of wastewater treatment and avert water crisis. However, in order to optimize biomass production in large-scale conditions, a detailed understanding of the metabolic response of the selected algal strain to the various conditions of light and temperature encountered over a year's operation should be done.

Declaration of competing interest

All authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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