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Domestic wastewater as nutrient media and its effect on biochemical composition of a marine diatom *Chaetoceros calcitrans*

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Abstract

Chaetoceros calcitrans was cultured in untreated domestic wastewater to evaluate its capacity to remove nutrients from wastewater as well as compare between the conventional wastewater primary treatment and microalgae treatment. Untreated and pre-treated domestic wastewaters were obtained from the primary wastewater treatment plant of the National Office of Electricity and Water in Rabat, Morocco. The experiments of microalgae treatment were carried out in our laboratory using Erlenmeyer flasks at room temperature (23-30°C) with an irradiance of 150 µmol photons and a light: dark cycle of 12:12 h. The maximum biomass (1.6 g L^{-1}) was obtained using raw domestic wastewater as culture medium compared to f/2 medium. Significantly higher carbohydrate (9.83%), lipid content (26.33%) and lower protein (31.60%) were obtained with raw domestic wastewater. High nutrient removal efficiencies were obtained using microalgae treatment for example 73.35% of total nitrogen (TN), 25.56% of total phosphate (TP) and 81.01% of chemical oxygen demand (COD) compared to conventional treatment (8.55, 21.28 and 73.91% respectively of TN, TP and COD). In addition, it was observed that SFAs increased and PUFAs decreased in raw domestic wastewater. These findings revealed that the marine diatom *C. calcitrans* has a high potential for use in wastewater treatment and biodiesel generation and can be a good candidate for the application of biorefinery system in Morocco.

Keywords: *Chaetoceros calcitrans*, domestic wastewater, microalgae treatment, conventional wastewater treatment, fatty acids

Full-length article **Corresponding Author*, e-mail: houria_elbakraoui@um5.ac.ma

1. Introduction

Human actions can have a detrimental effect on the environment. Any substance that can harm the environment is classified as a pollutant which can arise from a variety of sources, including human activities such as agriculture, land use change, production of waste, and others [1]. A significant part of human activities waste is domestic wastewater [2]. It was reported that more than 90% of clean water ends up as effluent [3]. Approximately 75% of domestic wastewater is created by residential structures or homes; the remainder is generated by office buildings, commercial areas, public facilities, and other sources [4]. Management of domestic management has traditionally been done in certain countries, for instance in Jakarta and Morocco, by dumping it into canals or rivers without any additional treatment or by utilizing septic tanks [4,5].

In 2020, Moroccan demands are estimated at 14.5 billion m^3 for agriculture alone and almost 1.75 billion m^3 for all other sectors [5]. By 2050, it will be 510 m^3 per person as predicted by the World Bank report on water resources. Over the past three decades, there has been a dramatic increase in the annual amounts of wastewater discharges. Between 1960 and 2005, they increased from 48 million to 600 million m^3, and by 2010, they had reached 700 million. These discharges

will continue to increase quickly, reaching 900 million m^3 in 2030 [6]. Since the 1960s, Morocco has established a water resources management policy by adopting laws 95/10 and 15/36 controlling water management systems in order to alleviate water stress and promote food security and socioeconomic growth. For wastewater discharged directly without any treatment into aquatic ecosystems, in particular the coast and rivers, Morocco has made great efforts in this sector by building 119 wastewater treatment plants with a treatment capacity of 405 m^3 d^{-1} [5]. Firstly, Morocco has used biotechnologies such as activated sludge, trickling filter, and biodisk to treat urban wastewater in various small and medium-sized cities, then the recent stations were created in 1990 and utilise advanced technologies such as natural lagoon basins [7].

Microalgae technologies (*Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Planothidium*, and *Synechococcus* genera) have mostly been used in wastewater treatment in Morocco for the biosorption of metals (Cd, Cr, Cu, and Zn) and the eradication of faecal coliforms, some pathogens (Salmonella, Vibrio cholerae), and helminth eggs [8]. Additionally, they used microalgae species, such as *Pseudokirchneriella subcapitata*, as integrated approach to assess the performance of municipal wastewater treatment plant [9]. Diatoms are one of the species that have been studied the most for determining the quality of water across the world because of their delicate time-dependent reaction [1]. They are the most group of photosynthetic eukaryotic microalgae found in practically all aquatic ecosystems with more than 10^5 extant species and being the only species containing silica in their cell [10]. In addition, they can fix 20% of global carbon and provide around 40% of primary marine productivity [11].

Diatoms have been identified as particularly interesting feedstocks for biodiesel synthesis due to their high biomass and lipid productivities in comparison to other algae species [12]. Even though producing cost-effective biofuels from diatoms will be challenging, an economically viable process will be more probable if importance is accorded to a number of high-value byproducts, such as wastewater treatment and the generation of bioactive substances [13]. Diatoms can multiply quickly, develop at great densities, absorb a lot of CO_2, nitrate, phosphate and produce a lot of oxygen. They can withstand a broad range of nutritional and environmental gradients, including sea ice, hot springs, fresh and hypersaline habitats, and different types of effluent. Due to their widespread prevalence and advantageous competitive characteristics, diatoms are excellent candidates for wastewater treatment [14]. One of the most significant diatoms, *Chaetoceros calcitrans*, is frequently used as live food in hatcheries for marine crustaceans, mollusks, and certain fish larvae and has a wide tolerance to temperature (10–35°C). In addition to providing animals with vital nutrients like polyunsaturated fatty acids, it is also helpful for treating aquaculture effluent and palm oil mill effluent but it has not been evaluated for domestic wastewater treatment [15]. It should be emphasized that the semi-arid environment of Morocco is ideal for the growth of *Chaetoceros calcitrans*. However, additional study on the design and operation of microalgae-based wastewater treatment processes is required in order for them to be more cost-effective and widely employed.

In the current study, the marine diatom *Chaetoceros calcitrans* was cultivated at room temperature (23-30°C) in a raw domestic wastewater obtained from the primary wastewater treatment plant of the National Office of Electricity and Water in Rabat, Morocco. The feasibility of raw municipal wastewater for the marine microalgae biomass growth, protein, carbohydrate, lipid production, fatty acids profils and nutrient removal were studied. The removal efficiency of microalgae treatment was also compared with the primary treatment of the wastewater treatment plant in Morocco.

2. Materials and Methods

2.1. Algae strain and preculture conditions

The marine diatom *Chaetoceros calcitrans* was obtained from the Moroccan National Institute for Halieutic Research collection. Before the experiment, the strain was maintained in standard f/2 medium [16] at room temperature (23-30°C) and under light intensity of 150 µmol photons with 12/12 h light/dark cycle.

2.2. Wastewater source

Raw and treated domestic wastewater were obtained from the primary wastewater treatment plant of the National Office of Electricity and Water in Rabat, Morocco. The samples of wastewater were transported to the laboratory, autoclaved at 121 °C for 20 min, and then kept at 4 °C until characterization and use. A pHmeter (Thermo ScientificTMOrion StarTM A111Benchtop pH Meter) was used to measure the pH and temperature. Turbidity and conductivity were measured using respectively a turbidimeter (2100N Laboratory Turbidimeter, EPA) and a conductimeter (Thermo Scientific STARA1120 Benchtop Conductivity Meter). Nitrate, total phosphate and chemical oxygen demand were determined using respectively the following colorimetric methods: sodium salicylate method [17], ascorbic acid method [18] and dichromate method [19]. Total nitrogen was measured using the TOC-V CPN coupled with ShimadzuTNM-1. The characteristics of raw and treated domestic wastewater are shown in Table 1.

2.3. Experimental procedures

Our study was based on the comparison of conventional wastewater primary treatment and microalgae treatment as shown in Fig. 1. The conventional primary treatment was actually done in the sewage primary treatment plant of the National Office of Electricity and Water in Morocco, while microalgae cultivation in domestic wastewater was examined in our laboratory as indicated below. *Chaetoceros calcitrans* cultivation was carried out in raw domestic wastewater using f/2 medium as control. The culture was performed in triplicate in 500 mL Erlenmeyer flasks with a working volume of 250 mL in a culture room temperature (23-30°C) with an irradiance of 150 µmol photons and a light:dark cycle of 12:12 h.

2.3.1. Biomass Growth Measurement

During 10 days of culture, microalgal growth was determined at days 0, 3, 5, 7 and 10 by measuring optical density at 680 nm using a spectrophotometer (Jasco V-730). The dry weight of *Chaetoceros calcitrans* was monitored using the following linear equation (E1) between biomass and optical density at 680 nm: (E1) $Y= 0.7633\ X + 0.016$ ($R^2 = 0.9893$), where Y is the dry weight (g L^{-1}) and X is the optical density at 680 nm. The specific growth rate µ (day^{-1}), doubling time G (day) and biomass productivity P (g L^{-1} day^{-1}) were calculated according to equations (E2, E3 and E4) [20]:

(E2) μ (day^{-1}) = ln(Y_1-Y_0)/ (t_1-t_0)

(E3) G (day) = ln(2)/μ

(E4) P (g L^{-1} day^{-1}) = (Y_1-Y_0)/ (t_1-t_0)

Where, Y_1 and Y_0 were the biomass concentration (g L^{-1}) on days t_1 and t_0, respectively.

2.3.2. Nutrient removal Measurements

In the final day of culture (day 10), samples were harvested by centrifugation at 8000 rpm for 10 min. The supernatant was analyzed for nitrate, phosphate and chemical oxygen demand using methods described in the section 2.2. The pellet was kept for biochemical analysis. Nutrient removal efficiency was determined using the following expression:

Nutrient removal efficiency (%) = $\frac{(C_0 - C_{10})}{C_0} * 100$

where C_0 is initial concentration of nutrient (mg L^{-1}) in supernatant and C_{10} is the final concentration (mg L^{-1}) after *Chaetoceros calcitrans* cultivation.

2.3.3. Biochemical Measurements

Lipid content of *Chaetoceros calcitrans* biomass was determined using the method of Bligh and Dyer [21]. 100 mg of microalgal dried biomass were maintained in 3.5 mL of a chloroform/methanol/water (2/1/0.5 v/v/v) mixture, vortexed for 2 min then centrifuged for 10 min at 8000 rpm. The organic phase was separated and placed in a tube that has been previously dried and weighed. The percentage of lipid (%) was calculated gravimetrically using equation

(5) Lipid percentage (%) = $\frac{Ml}{Mb} * 100$

where M_l is the mass of the extracted lipids and Mb is the mass of microalgal dried biomass.

For fatty acids analysis, 0.5 mL of chloroform was used to resuspend the extracted lipids. 0.1 mL of this samples were mixed with 0.8 mL of Boron trifluoride-methanol solution (10%) then heated to 100 °C in a water bath for 15 min. 0.75 mL of hexane and 1.5 mL of water were added and vortexed for 2 min. 10 µl of the upper phase was injected into an Agilent gas chromatography (6850) system to caracterize the methyl esters. Protein content was determined according to the method of Lowry et al. 1951 with the Folin phenol reagent [22]. In tubes, 0.5 mL of samples was combined with 0.7 mL of Lowry's reagent, vortexed, then incubated for 20 minutes at room temperature in the dark. Following that, 0.1 mL of Folin's reagent was added to the tubes, vortexed, and then placed in the dark for 30 minutes at room temperature. At 750 nm, the absorbance was measured using a spectrophotometer with Bovine serum albumin as a protein concentration standard for calibration curve.

Carbohydrates were determined according to the method of Dubois et al. 1956 [23]. 0.5 mL of phenol 5% (w/w) and 2.5 mL of sulfuric acid (>96%) were added to 0.5 mL of samples. The mixture was maintained at room temperature for 10 min, vortexed for 10 s, maintained for 15 min at room temperature, then incubated at 35°C in a water bath for 30 min. Total carbohydrates were calculated using the absorbance at 483 nm. Glucose was used as a carbohydrate standard.

2.4. Statistical analysis

All measurements were carried out in triplicate and the results were presented as mean value standard deviation. One-way analysis of variance was used to determine statistical significance at a level of $p < 0.05$.

3. Results and Discussion

3.1. Raw and pre-treated domestic wastewater characterization

Physicochemical characterizations of original and pretreated domestic wastewaters collected from the primary wastewater treatment plant of the National Office of Electricity and Water in Morocco are presented in Table 1. As shown in the table, raw and pretreated domestic wastewaters had high total nitrogen and total phosphate concentrations compared to f/2 medium. Algae growth requires a N/P ratio that is maintained between 6.8 and 10, but the wastewaters used in the study presented a critical N/P ratio of 2.29 which creates a stressful environment for lipids or carbohydrates accumulation by microalgae cells [24]. The chemical oxygen demand was observed to be higher in raw domestic wastewater of 442.72 ± 0.45 mg L^{-1} resulting in mixotrophic growth for microalgae cells. The composition of this wastewaters is consistent with that found in the literature [25], and it can be used to cultivate the microalga *Chaetoceros calcitrans* for two purposes: first, to treat raw domestic wastewater, and second, to produce lipids and other high value added compounds.

In general, wastewater is a source of a wide variety of microorganisms that, by competing for nutrients, might impede the growth of the desired microalgae [26]. In order to increase nutrient release, decrease microbial load, and reduce suspended particles in wastewater, several pre-treatment techniques (physical and chemical) have been shown to be helpful [25]. In the current investigation, the bacteria load in a raw domestic wastewater sample was reduced by autoclaving.

3.2. Biomass productivity and algal growth

The cultivation of *Chaetoceros calcitrans* during 10 days in raw domestic wastewater (RDW) and f/2 medium was conducted. The results of growth parameters (specific growth rate (μ)/doubling time (G)), biomass and biomass productivity are shown in Table 2 and Fig. 2. From the table, it is obvious that *Chaetoceros calcitrans* grow well in raw domestic wastewater in comparison to f/2 medium. The specific growth rate (μ) and the biomass productivity were significantly higher in raw domestic wastewater than that in f/2 medium about 0.484 ± 0.01 d^{-1} and 0.145 ± 0.003 g L^{-1} d^{-1} respectively in RDW compared to 0.209 ± 0.02 d^{-1} and 0.062 ± 0.001 g L^{-1} d^{-1} respectively in f/2 medium. As shown in Fig. 2, the biomass increased every day in both mediums (f/2 medium and raw domestic wastewater), but the RDW had a significant biomass production of 1.6 g L^{-1} compared to 0.77 g L^{-1} in f/2 medium.

Kumar Singh et al. (2021) cultivated *Chaetoceros sp.* and *Isochrysis sp.* in various concentrations of domestic wastewater (0-10-30-50-80 and 100%) diluted in seawater [27]. They found that culture of *Chaetoceros sp.* grown under 100% domestic wastewater revealed maximum biomass productivity of 2.79 g L^{-1} d^{-1} after 21 days of culture which is a high productivity compared to what we found but their results also confirm the increase in the productivity of the biomass in raw domestic wastewater [27]. According to research on *Phaeodactylum tricornutum* conducted by Wang et al. (2019), cells cultivated in culture using a 2:1 ratio of sewage treatment plant effluent and saltwater produced the highest levels of biomass concentration (1.021 mg L^{-1}) and productivity (0.289 g L^{-1} d^{-1}) at the final day of culture (day 10) [13].

Despite the fact that the wastewater used in the study had a critical N/P ratio of 2.29, which according to Katiyar et al. (2017) must be between 6.8 and 10 [24], the microalga *C.calcitrans* thrived in raw domestic wastewater. These findings might be attributed to organic nutrients in wastewater that acted as substrates for the production of *Chaetoceros calcitrans* biomass [28–30]. Sauvage et al. (2021) demonstrated that *Chaetoceros calcitrans* biomass can be increased up to a factor 2–2.5 under mixotrophic condition using glucuronic acid as substrate (analog of glucose) [30]. These results are similar to our finding indicated that *Chaetoceros calcitrans* grown in raw domestic wastewater produce 1.6 g L^{-1} de biomass compared to f/2 medium culture (0.77 g L^{-1}). The biomass increased with a factor of 2.1, suggesting that *C. calcitrans* is an excellent candidate for growth in raw domestic wastewater.

3.3. Removal efficiency of nutrients

The following parameters (total nitrogen, nitrate, total phosphate, and chemical oxygen demand) were measured on the first and last days of culture to examine the ability of *Chaetoceros calcitrans* to absorb nutrients from raw domestic wastewater. The removal efficiencies of TN, nitrate, TP and COD are shown in table 3. The finding affirm that *C. calcitrans* can remove 73.37%, 37.52%, 25.56% and 81.01% respectively of TN, nitrate, TP and COD from untreated domestic wastewater. Diatoms are helpful for bioremediation of a variety of heavy metals and can utilize nitrate, phosphate, iron, copper, molybdenum, and silica, but unlike green and blue-green algae, their potential for the treatment of wastewater has not yet been fully investigated [14,31]. A study investigated the viability of diatom cultivation in sewage wastewater in lab-scale trials, they discovered that the diatom consortium resulted in reductions of 95% of TN, 88.9% of TP and 91% of COD [32]. Their results is higher than our finding, but they used a consortium of diatoms instead of a single species which explains their highest removals. Recently, *Chaetoceros calcitrans* was co-cultivated with *Arthrospira platensis* on palm oil mill effluent (POME) under outdoor conditions. They revealed that 60% of COD can be removed by the mixture of microalgae from 30% of palm oil mill effluent, giving a specific growth rate of 0.4 d^{-1} and a final biomass of 0.440 g L^{-1}. The current research indicate that removal efficiency of chemical oxygen demand by *Chaetoceros calcitrans* from raw domestic wastewater was higher compared to Nur et al. finding [33].

Nutrients recovery rates by C.calcitrans in the current research from untreated domestic wastewater were higher than achieved in the study of Katiyar et al. (2021), where *Chlorella sorokiniana* removed 40 % of TN, 20.83 % of TP and 72.17 % of COD from municipal wastewater [34]. The highest removal efficiency of total nitrogen can be related to ammonia's simple assimilation by microalgae. Despite the fact that nitrate promotes the development of microalgae, those grown in wastewater first metabolize ammonia nitrogen, then nitrate nitrogen [35].

As shown in table 3, microalgae treatment removed 73.35% of TN compared to conventional primary treatment (8% of TN). The same pattern can be seen with nitrate, total phosphate and chemical oxygen demand respectively about 37.52%, 25.56% and 81.01% using microalgae treatment compared to 24.55%, 21.28% and 73.91% using conventional primary treatment. As a result, the present findings illustrate the effectiveness of microalgae nutrient absorption.

Traditional wastewater treatment employs a variety of physical, chemical, and biological processes and activities to remove particles, organic materials, and, in some cases, nutrients from wastewater [44]. Preliminary, primary, secondary, tertiary, and/or advanced wastewater treatment are general terminology used to describe different levels of treatment. Disinfection to eliminate pathogens is occasionally performed after the final treatment stage in some countries [45]. The primary wastewater treatment plant of the National Office of Electricity and Water in Rabat uses preliminary and primary treatment to remove particles and organic materials without secondary treatment. According to Asano et al, primary sedimentation removes around 50 to 70% of total suspended solids, some organic nitrogen, organic phosphorus, and heavy metals associated with solids, but colloidal and dissolved elements are unaffected [45]. In the current study, the removal efficiencies of nutrients using traditional primary treatment (8%, 21.28% and 73.91% respectively of TN, TP and COD) are in harmony with Asano et al. finding [45], but microalgae treatment proves its efficacy compared to conventional treatment and removes 73.35 of TN, 25.56% of TP and 81.01% of COD.

3.4. Effect on biochemical composition of Chaetoceros calcitrans

Microalgal biomass's biochemical content could vary depending on the strain and be affected by different growing circumstances and medium compositions [35–37]. To track changes in the content of proteins, carbohydrates, and lipids in the presence of wastewater, biochemical analysis of *Chaetoceros calcitrans* biomass were carried out and the results are presented in Table 4 and Fig. 3. Raw domestic wastewater and f/2 medium have diverse biochemical compositions, demonstrating the significant influence of culture media on *Chaetoceros calcitrans* metabolites. The highest lipid content of 26.33% was observed in raw domestic wastewater compared to f/2 medium (15.47%). It increased by 10.86%. Microalgae accumulate energy-rich substances such as lipids and carbohydrates under stressful growing circumstances [26]. The current study's findings revealed that raw domestic wastewater had a greater carbohydrate content of 9.83% than the f/2 medium (7.30%).

On the other hand, the control medium had the highest protein concentration of 38.43% in comparaison with raw domestic wastewater. Musetsho et al. cultured *Acutodesmus obliquus* on poultry litter and municipal wastewater mixture as nutrient and water sources and found that carbohydrate and lipid content were higher in the mixture compared to standard medium, but protein content was lower than BG11 medium [26]. Kumar et al. found that *Chaetoceros* sp. grown on f/2 medium had higher protein and carbohydrate content and lower lipid yield compared to raw municipal wastewater but *Isochrysis* sp. had lower protein and higher carbohydrate using f/2 medium compared to 100% municipal wastewater [27].

Table 1: Raw and pretreated domestic wastewater characteristics from the wastewater treatment plant.

Parameters	f/2 medium [13,16]	Raw wastewater	Pretreated wastewater (Conventional treatment)
pH	8.1	7.21 ± 0.02	7.53 ± 0.01
Temperature (°C)	nd	23.2 ± 0.30	23.4 ± 0.20
Turbidity (NTU)	nd	93.43 ± 0.65	11.86 ± 0.31
Conductivity (mS/cm)	nd	111.16 ± 1.03	90.80 ± 0.60
Total nitrogen (TN) (mg L^{-1})	12.4	26.82 ± 0.03	24.53 ± 0.02
Nitrate (mg L^{-1})	nd	3.26 ± 0.12	2.47 ± 0.05
Total phosphate (TP) (mg L^{-1})	1.2	11.69 ± 0.20	9.20 ± 0.03
Chemical oxygen demand (mg L^{-1})	nd	442.72 ± 0.45	115.51 ± 0.10

Nd: not determined

Table 2: *Chaetoceros calcitrans* growth characteristics in raw domestic wastewater compared to f/2 media (means ± standard deviations of three different tests, n = 3). Letters indicate statistical order of differences after one way ANOVA and post hoc tests ($p < 0.05$): a > b.

Medium	μ (d^{-1})	G (d)	Biomass productivity (g L^{-1} d^{-1})
f/2 medium	0.209 ± 0.02^{b}	3.31 ± 0.03^{a}	0.062 ± 0.001^{b}
Raw domestic wastewater	0.484 ± 0.01^{a}	1.43 ± 0.01^{b}	0.145 ± 0.003^{a}

Table 3: Removal efficiency of *Chaetoceros calcitrans* compared to conventional primary treatment of domestic wastewater (means ± standard deviations of three different tests, n = 3). Letters indicate statistical order of differences after one way ANOVA and post hoc tests ($p < 0.05$): a > b.

Parameters	Treated Domestic wastewater (Algal treatment) (mg L^{-1})	Algal Removal efficiency (%)	Conventional Removal efficiency (%)
Total nitrogen	7.15 ± 0.14	73.35 ± 0.55^{a}	8.55 ± 0.05^{b}
Nitrate	2.04 ± 0.03	37.52 ± 2.94^{a}	24.35 ± 3.01^{b}
Total phosphate	8.70 ± 0.10	25.56 ± 1.81^{a}	21.28 ± 1.48^{b}
Chemical oxygen demand	84.07 ± 1.01	81.01 ± 0.22^{a}	73.91 ± 0.02^{b}

Table 4: *Chaetoceros calcitrans* biochemical composition in raw domestic wastewater compared to f/2 media (means ± standard deviations of three different tests, n = 3). Letters indicate statistical order of differences after one way ANOVA and post hoc tests ($p < 0.05$): a > b.

Medium	Lipids (%)	Proteins (%)	Carbohydrates (%)
f/2 medium	15.47 ± 0.55^{b}	38.43 ± 0.21^{a}	7.30 ± 0.30^{b}
Raw domestic wastewater	26.33 ± 1.35^{a}	31.60 ± 1.31^{b}	9.83 ± 0.35^{a}

Table 5: Fatty acids profils of *Chaetoceros calcitrans* grown in raw domestic wastewater compared to f/2 media (means ± standard deviations of three different tests, n = 3). Letters indicate statistical order of differences after one way ANOVA and post hoc tests ($p < 0.05$): a > b. ND: Not Detected

Fatty acids profil	f/2 medium	Domestic wastewater
12:0 (Lauric acid)	ND	ND
14:0 (Myristic acid)	13.80 ± 0.10^{b}	22.30 ± 0.20^{a}
16:0 (Palmitic acid)	17.50 ± 0.50^{b}	19.80 ± 0.30^{a}
16:1n7 (Palmitoleic acid)	31.23 ± 0.05^{a}	29.93 ± 0.21^{b}
16:3n4 (Hexadecatrienoic acid)	15.23 ± 0.45^{b}	16.87 ± 0.15^{a}
18:0 (Stearic acid)	ND	ND
18:1n9 (Oleic acid)	0.60 ± 0.10^{a}	0.25 ± 0.05^{b}
18:3n3 (a-Linolenic acid)	0.70 ± 0.10^{a}	0.59 ± 0.06^{a}
18 :4n3 (Stearidonic acid)	0.23 ± 0.06^{b}	0.60 ± 0.10^{a}
20:1n9 (Eicosenoic acid)	ND	ND
20:3n6 (Eicosatrienoic acid)	ND	ND
20:5n3 (Eicosapentaenoic acid (EPA))	20.60 ± 1.14^{a}	14.95 ± 0.73^{b}
22:6n3 (Docosahexaenoic acid (DHA))	1.67 ± 0.15^{a}	1.64 ± 0.05^{a}
24:0 (Lignoceric acid)	ND	ND
Saturated fatty acids (SFAs)	31.30 ± 0.40^{b}	42.10 ± 0.44^{a}
Monounsaturated fatty acids (MUFAs)	31.83 ± 0.80^{a}	30.18 ± 0.18^{b}
Polyunsaturated fatty acids (PUFAs)	38.43 ± 1.45^{a}	34.65 ± 0.92^{b}

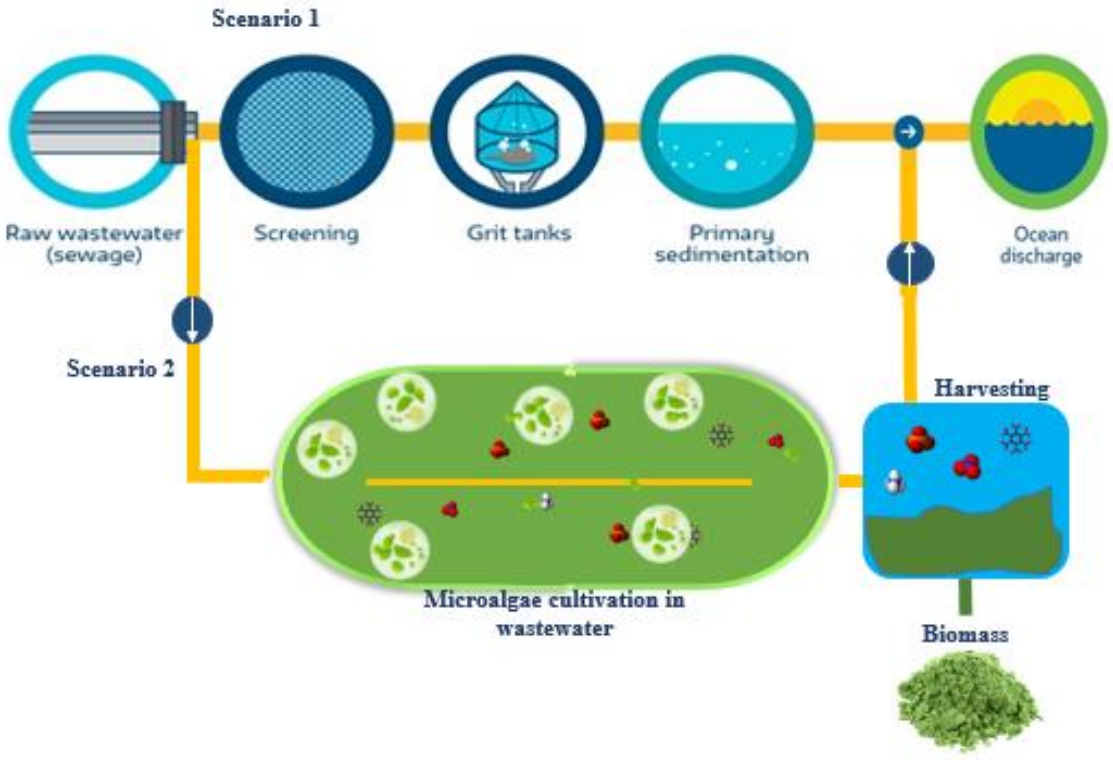

Fig. 1: Schematic diagram of the experiment

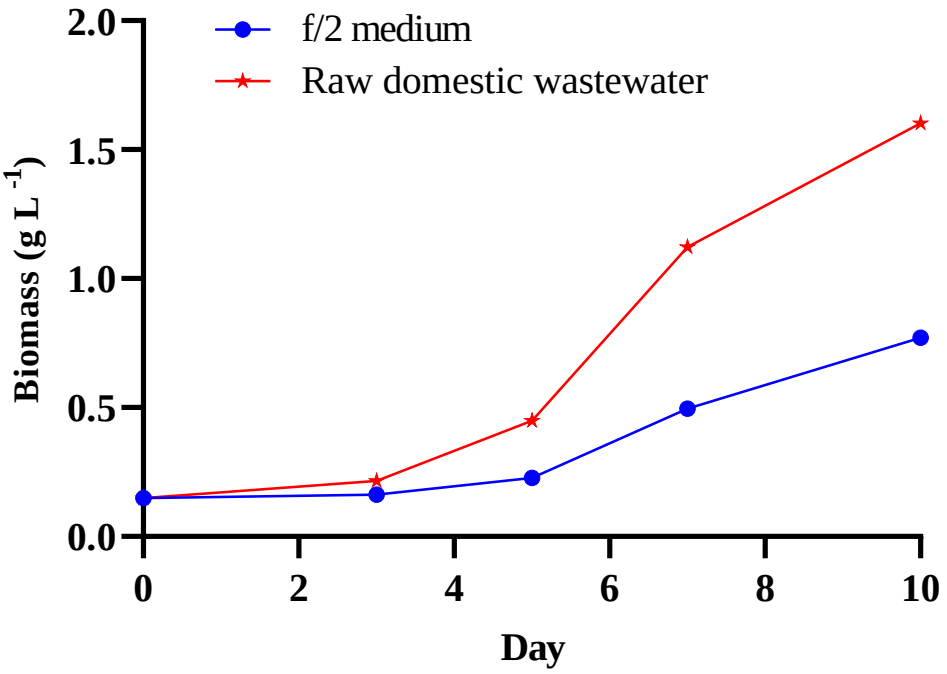

Fig. 2: Biomass of *Chaetoceros calcitrans* in raw domestic wastewater compared to f/2 media (means ± standard deviations of three different tests, n = 3).

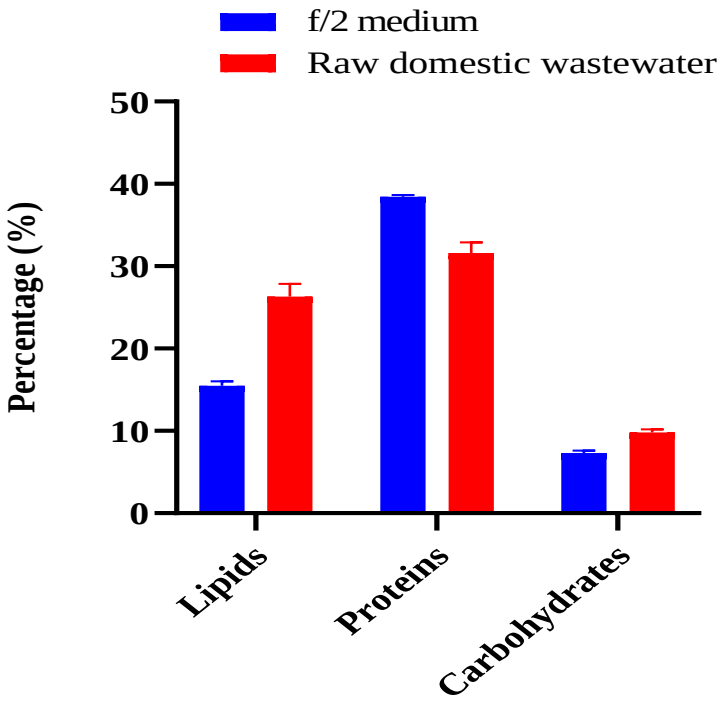

Fig. 3: Biochemical content (lipids, proteins and carbohydrates) of *Chaetoceros calcitrans* in raw domestic wastewater compared to f/2 medium

For lipid content of *Isochrysis* sp., there was no significant difference between 100% municipal wastewater and f/2 medium [27]. A study by Khatoon et al. was in contrast with our finding, indicated that lipid, protein and carbohydrate content of *Chaetoceros calcitrans* grown in aquaculture wastewater did not show any significant difference compared to Conway medium [31].

The findings of these investigations demonstrate that the biochemical composition of microalgae is particular to microalgae species and their culture conditions. Previous studies reported that wastewater's carbon source aids in the growth of microalgal biomass that is high in carbohydrates, while the availability of nitrogen, which microalgae typically use to make proteins, may account for the increased quantity of protein collected during autotrophic culture in synthetic medium [38,39]. In the current research, the highest lipid and carbohydrate content of *Chaetoceros calcitrans* grown in raw domestic wastewater may be due to the lowest N/P ratio of 2.29 which create a stressful environment according to Katiyar et al. (2017) [24].

3.5. Fatty acids profil of Chaetoceros calcitrans

It has been shown that each species of microalgae produces fatty acids with unique amounts and compositions [40]. Fatty acids composition of biodiesel of *Chaetoceros calcitrans* grown in raw domestic wastewater compared to f/2 medium is shown in Table 5. The content of Myristic acid and Palmitic acid was increased in raw domestic wastewater respectively from 13.80% to 22.30% and from 17.50% to 19.80%. These two saturated fatty acids (SFAs) are most prevalent, whereas the most abundant PUFA was 20:5n-3 (eicosapentaenoic acid-EPA). These findings are consistent with previous research indicating that diatoms have a very low content of C_{18} acid and a high content of EPA [41]. Saturated fatty acids represent 42.10% in biodiesel of *Chaetoceros calcitrans* grown in raw domestic wastewater compared to 31.30% in f/2 medium, but the content of monounsaturated fatty acids and polyunsaturated fatty acids was decreased in raw domestic wastewater. According to previous investigations, the increase of organic carbon content under mixotrophic conditions affected the fatty acid profile. Polyunsaturated fatty acids content of *Chlorella sorokiniana and Auxenochlorella protothecoides* decreased as the amount of glucose increased under mixotrophic conditions [42]. In addition, high culture temperature (23-30°C) can decrease the content of PUFAs and increased SFAs content in several species of marine microalgae [43]. Similar to our finding, a low proportion of PUFAs was reported by Ahmed Sas et al. in *Chaetoceros calcitrans* grown at 30°C [43].

4. Conclusions

Data show that *Chaetoceros calcitrans* may be successfully grown at room temperature on untreated domestic wastewater under artificial illumination yielding a maximum of biomass of 1.6 g L^{-1}. Additionally, when compared the removal efficiencies of nutrients of the primary treatment method (8.55% TN, 73.91% COD and 21.28% TP) and microalgae treatment, *Chaetoceros calcitrans* treatment exhibits better efficiency for the removal of nutrients (73.35% TN, 81.01% COD, and 25.56% TP). These findings might serve as a roadmap for the development of a *C. calcitrans* biorefinery system that treats wastewater while producing high-value-added products in regions with high temperature and light intensity, such as Morocco.

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