

Optimization of lab-scale harvesting-dewatering process of *Dunaliella salina* for industrial exploitation in Sabkhet Sidi El Heni saltworks, Tunisia

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ABSTRACT

Natural flotation of *D. salina* is uncommon phenomenon, but in saline environments, this microalga acquires a unique flotation capacity due to its high carotenoid and lipid content, giving it a lower density compared to the highly saline brine density. Laboratory-scale evaluations of this flotation capacity yielded promising results, with a concentration factor of 10.71, recovery efficiency around 37% and 100% cell integrity, values comparable to those obtained via other preconcentration techniques such as flocculation. This preconcentration step was followed by a dewatering phase through centrifugation. Direct centrifugation alone was ineffective due to the high brine salinity and *D. salina* hydrophobicity, necessitating a salinity reduction step before optimal dewatering. Using single-factor and Box-Behnken design experiments, dewatering was optimized by studying centrifugation speed, salinity, and time, with centrifugation speed proving most influential. Optimal conditions (6500 rpm, 10 minutes, and 70 g/L salinity) resulted in 95.79% cell integrity and 98.79% recovery efficiency. These findings suggest that sequential preconcentration via flotation, followed by dilution and centrifugation, provides a sustainable method for saline environments, significantly enhancing both cost and energy efficiency.

Keywords: *Dunaliella salina*, harvesting-dewatering process, natural flotation, centrifugation, Response surface methodology, carotenoids.

INTRODUCTION

Dunaliella salina is a halophilic microalga thriving in saline environments, with applications in pharmaceuticals, nutraceuticals, food, and cosmetics due to its rich β -carotene content, a precursor of vitamin A, renowned for its potent antioxidant properties (Ben-Amotz et al., 1982; Yarkent et al., 2020). Under stressful conditions, such as high light intensity, high temperatures, nitrogen deficiency, and salinity exceeding 70 g/l, *D. salina* can produce β -carotene and lipids (Hosseini and Shariaty 2009). Consequently, the mass cultivation of *Dunaliella* in pilot ponds worldwide holds promise for economically viable production, particularly in the pharmaceutical,

nutraceutical, cosmetic, and bioenergy sectors (Shariaty and Hadi, 2011).

Harvesting microalgal biomass is a crucial step in various industries (Ortiz et al., 2021), with success dependent on several factors, including the species studied, cell size, biomass density in the culture medium, and the value of the desired final product (Rawat et al., 2011). Separation of *D. salina*, in particular, poses significant challenges. Since *D. salina* lacks a rigid cell wall, it is fragile and sensitive to shear forces, making it prone to rupture under high shear stress conditions. Moreover, its culture is typically diluted (~0.5 g/l), and its density varies depending on culture conditions, usually around 1.1 g/l, which complicates easy decantation (Misra et al., 2015). These parameters make the separation of *D. salina* one of

the main obstacles to its production development, necessitating the employment of suitable harvesting methods to address these challenges.

SAIDA S.A. is one of the companies aiming to capitalize on the potential of *D. salina*. It operates Sabkhet Sidi el Heni lagoon for salt extraction and is currently conducting a pilot project for the extensive exploitation of *D. salina* to supply biomass to various industries that value β -carotene in cosmetic formulations, nutraceuticals, and pharmaceuticals. This initiative enables the company to harness the bioresources present in the brines, fostering support for a sustainable economy alongside salt extraction. However, one of the main challenges faced by SAIDA S.A., like all biorefineries exploiting microalgae, lies in the harvesting and dewatering stages. In saltworks, *D. salina* cells remain neutrally buoyant due to the high specific gravity and viscosity of the brine. Given that cell densities in large-scale cultures are low, processing extremely large volumes of culture is necessary to efficiently harvest the biomass (Ben-Amotz, 2020). Moreover, the company must find a reliable and cost-effective method that avoids using chemicals which could compromise the quality of the final product or interfere with salt production. This is particularly important in Tunisia since saltworks products must meet organic standards as stipulated by Article 14 of the Tunisian Mining Code (Mine code of Tunisia, 2017). Additionally, preserving the cellular integrity of the microalgae is crucial to maintaining their carotenoid content, which is highly susceptible to oxidation (Gultom and Hu, 2013).

Several methods for harvesting and dewatering *D. salina* biomass have been reported, including membrane filtration (Monte et al., 2018; Monte et al., 2020), flocculation (Xiong et al., 2015), centrifugation (Yaich-Achouri et al., 2021), and flotation (Javed et al., 2019). Each of these techniques has its own set of advantages and disadvantages.

For instance, centrifugation is a widely used method for harvesting *D. salina* due to its high efficiency in separating highly diluted solutions, recovering up to 90% of the biomass in a relatively short time (Najjar and Abu-Shamleh, 2020). A key advantage of centrifugation is that it does not require chemical additives, which helps maintain the quality of the harvested biomass over time. Therefore, it is particularly suitable for applications where high purity is essential, such as in the pharmaceutical and food industries. However,

centrifugation also has significant drawbacks, notably its high energy consumption and operational costs, which can make it less viable for large-scale operations or low-value products like biofuels, where economic efficiency is critical. Also, at high rotational speeds, centrifugation can damage the cells, leading to the loss of valuable compounds (Besson et al., 2019), necessitating the optimization of parameters to ensure efficient and gentle biomass recovery (Najjar and Abu-Shamleh, 2020). We should also note that the success of the centrifugation process depends on the final outcome in terms of recovery efficiency and the preservation of the microalgal biomass's cell integrity. Several parameters can influence this process, including centrifugation speed, duration, and other factors specific to each microalga and experiment.

Conversely, membrane filtration, especially ultrafiltration, presents an alternative with several advantages. It allows for the preconcentration of microalgae, significantly reducing energy consumption and operational costs, particularly when combined with centrifugation. This combination can reduce energy consumption by up to 76%, making it a more cost-effective option (Najjar and Abu-Shamleh, 2020). Furthermore, controlled transmembrane pressure in ultrafiltration allows for the concentration of cells while preserving their integrity. However, membrane filtration poses its own challenges, such as the risk of membrane clogging due to the small size of *D. salina* cells and the exopolysaccharides they secrete (Uduman et al., 2010). This issue can increase maintenance requirements and operational costs, especially in long-term or large-scale operations (Borowitzka and Borowitzka, 1990).

On the other hand, flocculation, a low-cost method suitable for large-scale harvesting, presents another alternative to centrifugation and membrane filtration. This technique uses flocculants to aggregate microalgae cells, making them easier to separate from the culture medium (Garg et al., 2012). While economically attractive for large-scale operations, the use of synthetic flocculants raises concerns about contamination, as these chemicals can remain in the harvested biomass and interfere with downstream applications, particularly in the food, feed, cosmetic, nutraceutical industries, and recycled water (Gerardo et al., 2015). Moreover, flocculation may not achieve the same level of efficiency as centrifugation or membrane filtration in applications requiring high purity.

In summary, centrifugation is considered the most reliable harvesting method that meets most of SAIDA S.A.'s requirements. However, its energy-intensive nature directly impacts production costs. To mitigate this difficulty, a preconcentration step before centrifugation can help reducing energy consumption (Demir et al., 2020).

Preconcentration methods such as flocculation, and flotation have primarily been applied to *D. salina* cultures in controlled environments like raceways, where harvesting techniques are generally mechanized or chemical-based. In contrast, harvesting microalgae in inland salinas, a more cost-effective production method, often relies on environmental conditions such as high temperatures, wind, and sunlight. These methods utilize natural forces like flotation, evaporation, and adsorption and are based on simple, unsophisticated techniques.

Borowitzka and Borowitzka, 1990 highlighted several harvesting methods that had already been tested and found applicable in salt lagoons by leveraging the physical and morphological characteristics of *D. salina*. These methods include the use of salinity-dependent buoyancy properties in both stationary and moving gradients (Bloch et al., 1982), the exploitation of phototactic and gyrotactic responses to enable more precise collection (Kessles et al., 1985), and the application of salinity-dependent hydrophobic adhesion properties to improve harvesting efficiency (Curtin and Snook, 1983).

One such natural phenomenon appeared spontaneously at Sabkhet Sidi el Heni lagoon, where a natural preconcentration phenomenon of *D. salina* occurred along the banks of the salt crystallization basins due to extreme climatic conditions that facilitate its flotation. Few studies have reported on the natural flotation capacity of *D. salina* (Bloch et al., 1983; Borowitzka and Borowitzka, 1990), which has yet to be effectively harnessed for efficient harvesting. Nonetheless, this technique has proven effective for harvesting other microalgae, exploiting their natural characteristics, such as their relatively low density, which allows them to float (Singh and Patidar, 2017). Thanks to its relatively rapid operation, low space requirements, high flexibility, and moderate operational costs, flotation holds promise for addressing challenges in microalgal exploitation and biomass production (Yang et al., 2001).

This observation prompted the scientific team at SAIDA S.A. to explore this phenomenon on a laboratory scale, aiming to achieve natural

preconcentration of *D. salina*, followed by a dewatering step via centrifugation.

Numerous studies have highlighted the efficacy of these two methods in harvesting microalgae individually. Thus, their combined use allows for the reduction of energy consumption while maintaining the resulting biomass quality (Yang et al., 2001). However, no studies have yet pinpointed the optimization parameters influencing *D. salina* biomass quality (cell integrity) and separation yield (recovery efficiency). Therefore, this study investigates the preconcentration phenomenon along the banks of saltworks through the natural flotation of *D. salina* and explores biomass dewatering via laboratory-scale centrifugation. We also examine the combined effects of these two methods on recovery efficiency and cell integrity of the recovered biomass, with optimization of the centrifugation process using response surface methodology (RSM). This approach allows for the simultaneous analysis of the effects and interactions of various factors on cell integrity and recovery efficiency, thus optimizing factors that impact energy consumption and cost management (Abu-shamleh and Najjar, 2020).

The findings of this study will guide subsequent downstream processing steps to obtain biomass at preindustrial and industrial scale.

EXPERIMENTAL SECTION

Microalgal source

The native *D. salina* was isolated from the salt crystallisation basins edges of Sabkhet Sidi El Heni, in inland salina located in the central region of Tunisia. Sabkhet sidi el henii is a NW–SE lengthened depression in the Sahel area (Latitude : 35° 32' 57" (35,5492°) north, Longitude : 10° 26' 57" (10,4492°) east. At the end of June 2020, a phenomenon of natural preconcentration of *D. salina* (Figure 1) occurred on the edges of salt crystallization basin N°7 due to several extreme climatic conditions, including Temperature (3–48 °C), luminosity (592–1975 $\mu\text{mol/s/m}^2$) during 15 hours a day, salinity (320–370 g/l), and mainly wind movement. A homogeneous sample of 10 L of *D. salina* preconcentrate with a pH of 7.2 and a high cell concentration of 79.33×10^6 cells/ml was recovered in order to obtain *D. salina* paste. Separation of *D. salina* from Sabkhet Sidi El Heni was explored following the schematic process (Figure 2).



Figure 1. Natural preconcentrate

Harvesting procedure

Analytical methods

The optical density was measured by the mean of a spectrophotometer ; cell counting and cell integrity (CI) were determined by a microscopic observation using a cell couter (malassez cell). The percentage was calculated using the following equation:

$$CI (\%) = \frac{\text{Number of intact cells}}{\text{Total number of cells}} \times 100 \quad (1)$$

The salinity was measured by the mean of a refractometer. The recovery efficiency (RE) and

the concentration factor (CF) were determined according to the following equations, respectively:

$$RE = \left(1 - \frac{n_e}{n_i}\right) \times 100 \quad (2)$$

$$CF = \frac{DO_i V_i - DO_e V_e}{DO_i V_f} \times 100 \quad (3)$$

where: n_i – number of algal cells in the initial suspension; n_e – number of algal cells in the depleted phase; DO_i – Optical density at 760 nm in the initial suspension; DO_e – Optical density at 760 nm in the depleted phase; V_i – volume of the initial suspension (ml); V_f – Concentrated phase volume (ml).

The centrifugation procedure was carried out using a laboratory centrifuge (MPW-280/ Max :14000 rpm).

Natural flottation

The cell density of the sample taken from the salt ponds (natural preconcentrate) was determined through microscopic counting using a Malassez cell, and subsequently confirmed by spectrophotometric measurements. Due to the sample’s very high cell concentration, several dilutions were performed to enable accurate counting and optical density measurement. The final result was multiplied by the corresponding dilution factor, yielding a concentration of 79.33

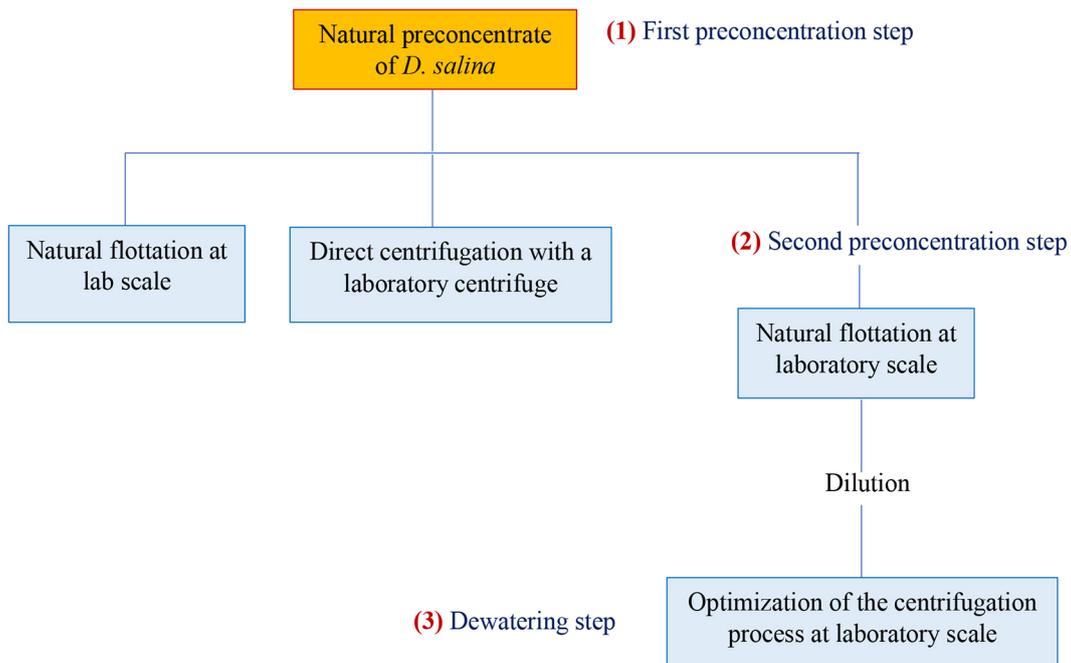


Figure 2. Workflow diagram

$\times 10^6$ cells/ml. The flotation capacity of *D. salina* was assessed in triplicate using 250 ml separating funnels filled with 150 ml of *D. salina* suspension (Figure 3). The funnels were left for 6 hours at room temperature under artificial light using fluorescent tube (40 watts), after which the concentration factor (CF) and recovery efficiency (RE) were determined using the Equation 2 and 3.

Direct centrifugation of *D. salina* preconcentrate

The dewatering process of *D. salina* natural preconcentrate was carried out using a centrifuge (MPW-280 Lab centrifuge/Max: 14000 rpm). 40 ml of the algal suspension was put into 50 ml conical bottom flask. Then the separation was carried out at various centrifugation speeds (4000; 5000; 6000; 7000; 8000; 9000 and 10000 rpm) during 15 minutes.

Experimental design of *D. salina* dewatering by centrifugation

The microalgal preconcentrate was firstly re-concentrated by a natural flottation. The harvested biomass was then recovered and used to perform the rest of experiments. Salinity control was achieved by diluting the recovered biomass with distilled water.

- single factor design – initially, a single-factor test was employed to establish the preliminary ranges for the centrifuge separation variables. Each wet biomass underwent separation with designated salinity, time, and centrifugation speed. One factor was modified at a time while keeping the other factors constant (Figure 4).

- Box-benhken design – based on preliminary test results, three variables: centrifugation speed (RPM) (ranging from 5000 to 8000 rpm), suspension salinity (ranging from 40 to 105 g/l) and time (ranging from 5 to 15 min), and, designated as X_1 , X_2 , and X_3 , respectively, were investigated by Box-Behnken Design (BBD) using Statgraphics (version 17.1.12) to assess recovery efficiency (Y_1) and cell integrity (Y_2). The results including the complete design comprised 17 experimental points are displayed in Table 1. To estimate the pure error, 5 repetitions were strategically placed at the design center. To forecast the ideal conditions, we used an empirical second-order polynomial model and adjusted the regression coefficients to establish connections between variables and responses according to the following equation

$$Y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum_{i < j=2}^k \beta_{ij} X_i X_j \quad (4)$$

Y represents the dependent variable (Y_1 and Y_2 are the predicted recovery efficiency and cell integrity of *D. salina* cells, respectively), where k is the number of variables ($k = 3$ in this experiment); β_0 is the model intercept; β_j , β_{jj} and β_{ij} represent the coefficients of the linear terms, quadratic terms, and interaction terms, respectively. X_i and X_j are the actual values of independently coded variables ($i \neq j$) (Samavati and Yarmand, 2013). The results were confirmed by the presence or absence of

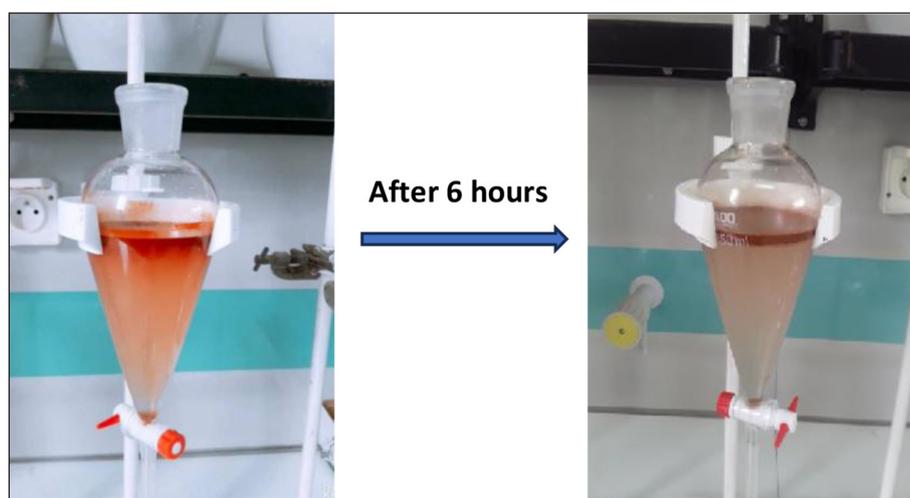


Figure 3. Lab scale natural flotation of *D. salina*



Figure 4. Recovery of *D. salina* paste by centrifugation after dilution

Table 1. Box–Behnken experimental design and results for recovery efficiency (%) and cell integrity (%) of *D. salina*

Run	X_1	X_2	X_3	Response	
	Speed (rpm)	Salinity (g/l)	Time (min)	Recovery efficiency (%)	Cell integrity (%)
1	6500	72.5	10	97	95
2	6500	72.5	10	96	96
3	6500	72.5	10	95	97
4	6500	72.5	10	97	95
5	6500	72.5	10	97	96
6	5000	72.5	15	54	85
7	5000	72.5	5	43	98
8	8000	105	10	95	52
9	6500	105	15	87	83
10	6500	105	5	74	96
11	6500	40	5	82	96
12	6500	40	15	97	85
13	5000	105	10	45	97
14	8000	40	10	98	51
15	8000	72.5	5	83	59
16	8000	72.5	15	99	42
17	5000	40	10	52	97

carotenoids determined by UV spectrophotometry in the supernatant after centrifugation. A water solution devoid of carotenoids served as negative control for the UV carotenoid determination.

Carotenoids content

The results were confirmed by quantification of the total carotenoids content in the recovered fresh paste. Pigments were extracted from *D. salina* fresh biomass and quantified spectrophotometrically. A total of 10 mg dry algae biomass

was dispersed in 0.5 mL acetone and disintegrated with 0.5 mL and vortexed for 15 min, then the extract was separated through centrifugation and collected. The extraction step was repeated until the biomass pellet was decolorized. The extracts were combined and made up to 10 mL in volumetric flasks.

The absorbance of the appropriately diluted extracts was measured at 470, 647 and 662. The pigment contents were determined according to the equation provided by Lichtenthaler (1987)

considering the dilution factor and expressed in (% w/Fw).

$$\begin{aligned} \text{Chla (mg ml}^{-1}\text{)} &= \\ &= 11.24 \times A_{662} - 2.04 \times A_{645} \end{aligned} \quad (5)$$

$$\begin{aligned} \text{Chlb (mg ml}^{-1}\text{)} &= \\ &= 20.13 \times A_{645} - 4.19 \times A_{662} \end{aligned} \quad (6)$$

$$\begin{aligned} \text{Cart (mg ml}^{-1}\text{)} &= (1000 \times A_{470} - 1.9 \times \text{Chla} - \\ &- 63.14 \times \text{Chlb})/214 \end{aligned} \quad (7)$$

where: A_{662} – absorbance measured at 662 nm, A_{645} – absorbance measured 645 nm, A_{470} – absorbance measured 470 nm

Statistical analysis

Experimental data for carotenoid content in *Dunaliella salina* recovered paste are expressed as mean \pm standard deviation (SD). For each recovery condition, carotenoids were measured in triplicate from the same sample. The different recovery conditions corresponded to independent trials. Statistical differences among trials were evaluated using one-way ANOVA, followed by Tukey's HSD test for multiple comparisons. Differences were considered significant at $p < 0.05$. All analyses were performed using XLSTAT 2024.

RESULTS AND DISCUSSION

Natural flottation

Understanding the natural preconcentration phenomenon of *D. salina* in salt pans

Sabkhet Sidi El Heni, a lagoon in central Tunisia, lies atop extensive salt tables, making it ideal for salt production. Its harsh climate, characterized by prolonged, hot summers, and high salinity create an optimal environment for the growth of *D. salina*, known for its richness in β -carotene. These conditions contribute to a phenomenon of natural preconcentration along the banks of the salt basins, occurring over multiple cycles throughout the summer.

Previous studies, primarily conducted in raceway ponds, revealed that *D. salina* is typically unable to float naturally (Demir et al., 2020). However, in high-salinity salt extraction ponds at Sabkhet Sidi El Heni salinas, the microalga demonstrates buoyancy. This phenomenon was previously evoked by Borowitzka and Borowitzka, (1990), who explained that *D. salina* can float on the salt pond surface due to the formation

of a low-salinity layer after rainfall. The cells accumulate at the interface between the low- and high-salinity layers, allowing them to remain buoyant (Borowitzka, 2005).

Considering this explanation, we can deduce that at Sabkhet Sidi El Heni, where salt accumulates at the bottom of the basins, the brine becomes relatively less saturated as it rises to the surface. This gradient promotes the accumulation of *D. salina* at the top, allowing the cells to gather in the less dense, upper layers. Additionally, the cells exhibit diurnal movement, with increased day length and light intensity promoting greater surface aggregation (Wangersky and Maass, 1988). The natural air currents in the area further assist the wind in moving these aggregates toward the basin banks.

Interestingly, cells with the highest β -carotene content tend to stratify more easily, allowing a natural selection process that differentiates mature cells ready for harvest from those still developing (Borowitzka, 2005). This phenomenon presents a potential advantage for the efficient production and collection of *D. salina*, serving as a preliminary step in the harvesting process (Borowitzka, 2005).

D. salina lab scale flottation

After six hours of settling in separatory funnels under artificial light, *D. salina* floated to the surface, achieving a concentration factor of 10.71 and a recovery efficiency of 37%, while maintaining 100% cell integrity. The cells were viable, in good condition, and exhibited moderate mobility. Indeed, under sufficient light and without agitation, viable *D. salina* cells naturally float to the surface to seek light.

The high salinity concentration (>320 g/L), which increases the medium's density (Kim et al., 2005), combined with the abundance of lipids and carotenoids in *D. salina*, enhances its hydrophobicity and facilitates surface flotation (Garg et al., 2015). Curtin (Curtin, 2000) reported that brine salinity levels in Australian *D. salina* ponds, reaching up to 320 g/L, increased the hydrophobicity of the microalgal cell membrane, a phenomenon less pronounced at salinity levels around 170 g/L. Borowitzka (2005) and Ben-Amotz et al., (2009) further confirmed that *D. salina* becomes neutrally buoyant in high-density, high-viscosity brine. Furthermore, the extreme environmental conditions of Sabkhet Sidi El Heni, characterized by high salinity and luminosity, have led to a change

in the behavior of the microalga compared to the same species cultivated in artificial raceways with much lower salinity, resulting in increased surface flotation activity (Besson et al., 2019).

We note that the concentration factor detected in this study is comparable to that reported by Monte and his colleagues (Monte et al., 2018) who achieved similar results using membrane filtration to harvest *D. salina*, with slightly lower cell integrity (95%). This comparison highlights the effectiveness of the flotation, which proves to be a more cost-efficient option than the membrane filtration, particularly in terms of operational and maintenance expenses.

We deduce that natural flotation is a good solution for preconcentrating *D. salina* biomass, maintaining cell integrity, and preserving valuable compounds. Additionally, this method is both cost-effective and free of chemicals, making it ideal for maintaining the quality of the recovered biomass and salt production in Sebkhah of Sidi El Heni salt pans. The residual suspension, free of chemicals, contains immature *D. salina* cells that have not yet reached optimal β -carotene production and lack the hydrophobicity needed to float. These cells can be returned to the salt ponds to support further growth and increase β -carotene accumulation (Borowitzka and Borowitzka, 2005).

However, improvements in flotation capacity could enhance recovery efficiency in downstream processes. This method can also be applied to harvest *D. salina* from areas of salt ponds with lower cell concentrations. Nonetheless, the method alone is insufficient for effective dewatering, requiring an additional centrifugation step to improve biomass quality.

D. salina harvesting by centrifugation

The influence of centrifugation speed on *D. salina* recovery and cell integrity is presented in Table 2. Results indicate incomplete separation of *D. salina* biomass, forming a creamy layer at the surface in all tests at various centrifugation speeds. This suggests that the gravitational force may not be sufficient to sediment *D. Salina* cells. Additionally, red water is observed below the creamy phase of *D. salina*, indicating the presence of either intact cells that were not recovered or released carotenoids following cell lysis due to strong mechanical agitation.

This atypical behavior is likely due to the high salinity of the suspension, as the solution density increases with salinity, while the hydrophobic nature of red *D. salina*, rich in carotenoids and lipids, makes it less dense and tends to float on the surface. Such behavior has not been mentioned in the literature before, highlighting the unique characteristics of *D. salina* of Sabkha of Sidi El Heni, in response to centrifugal force.

This finding can be explained by the fact that salinity concentration at Sebkhah is higher (saturated brines) than that generally used for the artificial growth of *D. salina* in raceways. Therefore, dilution is necessary to reduce salinity and precipitate *D. salina* during centrifugation. Regarding the integrity of *D. salina* cells, we note a variation depending on the centrifugation speed, with maximum integrity observed at 4000 rpm and decreasing gradually to 50% at 8000 rpm. The current results suggest that optimizing the separation of the microalga by centrifugation requires careful consideration of salinity and centrifugation speed. Additionally, the centrifugation time

Table 2. Impact of direct Centrifugation of *D. salina* suspension on recovery efficiency and cell integrity

rpm	Appearance	Cell integrity (%)
4000	- Appearance of a creamy layer of <i>Dunaliella</i> at the surface - The water volume remained below	100
5000	- Appearance of a creamy layer of <i>Dunaliella</i> at the surface - The water volume remained below	95
6000	- Appearance of a creamy layer of <i>Dunaliella</i> at the surface - The water volume remained below	80
7000	- Appearance of a creamy layer of <i>Dunaliella</i> at the surface - The water volume remained below	65
8000	- Appearance of a creamy layer of <i>Dunaliella</i> at the surface - The water volume remained below	50
9000	- Appearance of a creamy layer of <i>Dunaliella</i> at the surface - The water volume remained below	50
10 000	- Appearance of a creamy layer of <i>Dunaliella</i> at the surface - The water volume remained below	47

factor is essential to study, as prolonged exposure to physical force may lead to cell lysis.

Experimental design of *D. salina* harvesting by centrifugation

In this section, we present the results of optimizing the parameters that influence the recovery efficiency and cellular integrity during the recovery of *D. salina* biomass by centrifugation, namely salinity, centrifugation speed, and centrifugation time. A preliminary step of biomass preconcentration using natural flotation was conducted to counteract biomass loss due to dilution and to remove as much brine as possible. Salinity adjustment was achieved by dilution of the preconcentrated biomass with freshwater.

Single factor experimental design

Effect of the centrifugation speed variation

Only the centrifugation speed was varied (ranging from 3000 to 12000) while the other parameters salinity, and time were fixed at 63 g/l and 10 min, respectively (Figure 5a). We notice that recovery efficiency increases gradually reaching its highest level (97%) at 12000 rpm, while the cell integrity decreases with increasing the centrifugation speed. The highest cell integrity value (100%) was registered at 3000 and 4000 rpm. Therefore the centrifugation speed ranging from 5000 to 8000 rpm was selected for further BBD experiments.

Effect of varying salinity concentration

The effect of various salinity concentration ranging from 1 to 320 g/l was illustrated in Figure 5b, while the other conditions (centrifugation speed and separation time) were kept constant at 6000 rpm and 10 minutes, respectively. The results show that the cell integrity is not influenced by salinity variations. However, the recovery efficiency decreases gradually with increasing the salinity, reaching a minimum value (4%) at the highest tested salinity concentration (320 g/l). Consequently, the salinity concentration range from 4 to 105 g/l was selected for the further BBD experiments.

Effect of varying centrifugation time

The recovery efficiency and cell integrity under different centrifugation times were shown in Figure 5c. All the other conditions were

maintained constant (salinity 63 g/l and centrifugation speed 6000 rpm). We notice that recovery efficiency increases with increasing the centrifugation time until reaching its maximum level (91%) after 25 minutes. Conversely, cell integrity is negatively influenced by centrifugation time. This is can be attributed to the fragility of the cell membrane of *D. salina* which cannot withstand prolonged physical force. Consequently, the separation time of 5 to 15 min was considered for further BBD experiments.

Optimization of *D. salina* separation by BBD

Model fitting and statistical analysis

Based on the multiple regression analysis derived from the BBD experimental data, the relationship between response variables and test variables were expressed by the following second-order polynomial equation:

$$Y_1 = -210.358 + 0.103689 \times X_1 + 3.35974 \times X_3 + 0.168619 \times X_2 - 0.00000901111 \times (X_1)^2 - 0.000133333 \times X_1 \times X_3 + 0.00000512821 \times X_1 \times X_2 - 0.181 \times (X_3)^2 - 0.00307692 \times X_3 \times X_2 - 0.0012071 \times (X_2)^2 \quad (8)$$

$$Y_2 = -406.712 + 0.125029 \times X_1 + 6.17474 \times X_3 + 0.383471 \times X_2 - 0.0000087 \times (X_1)^2 + 0.000166667 \times X_1 \times X_3 + 0.0000205128 \times X_1 \times X_2 - 0.283 \times (X_3)^2 - 0.00307692 \times X_3 \times X_2 - 0.00409467 \times (X_2)^2 \quad (9)$$

The experimental data were analyzed by ANOVA to assess the models' significance, then to evaluate the adequacy and the fitness of the models. Table 3 depicted the summarized results.

F- and *p*-values were leveraged for assessing the statistical significance of each coefficient within the model. The model *F*-values, 18.55 with a low *p*-value of 0.0021 for recovery efficiency, and 26.45 with a *p*-value of 0.0003 for cell integrity, denote the high significance of the regression models. *P*-values were further deployed to evaluate the significance of the linear coefficients (X_1, X_2, X_3), the quadratic term coefficients (X_1^2, X_2^2, X_3^2) and the interaction coefficients (X_1X_2, X_1X_3, X_2X_3) (Table 3). All linear values linked to centrifugation speed (X_1), salinity (X_2), and time (X_3) and their quadratic values were found to be highly significant ($P < 0.05$) for recovery efficiency. This observation underlines the important role of all analyzed variables in this study as significant factors which independently influencing the recovery efficiency of *D. salina* cells.

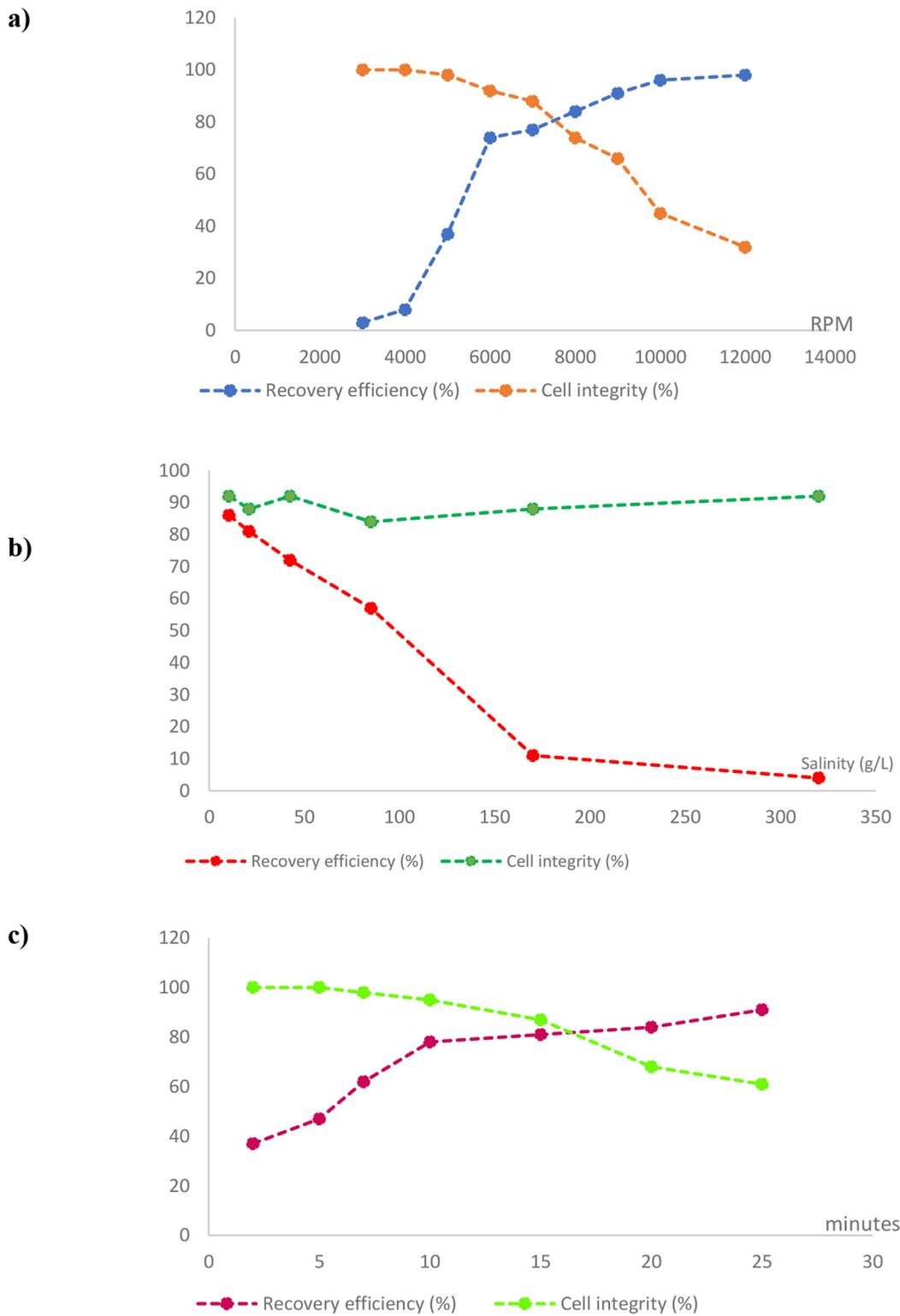


Figure 5. Effect of centrifugation speed (a), salinity (b) and separation time (c) variations on the recovery efficiency and the cell integrity of *D. salina* paste

The linear coefficients related to the cell integrity response (X_1 and X_3) and the quadratic coefficients (X_1^2 and X_2^2) exhibited significant effects ($p < 0.05$). Whereas, other coefficients (X_2 ,

X_1X_2 , X_1X_3 , X_2X_3 and X_3^2) were considered statistically insignificant ($p > 0.05$).

For assessing the model's applicability, determination of coefficients (R^2) and adjusted

determination coefficients (R^2_{adj}) were employed. The R^2 and R^2_{adj} values associated to the recovery efficiency response were 0.9921 and 0.9908, respectively. Those related to the cell integrity were 0.9951 and 0.9824. These findings indicate a satisfactory correlation between the experimental and the response variables.

Analysis of response surfaces: Three demensional response surface

The three-dimensional (3D) response surface depicted interactions between the variables, helping to identify the maximum, minimum, and saddle points of the responses. For each surface, the dependent variable (Y_1 or Y_2) is represented as a function of two parameters, while the third is maintained at its zero level.

Figures 6a and 6b demonstrate the three-dimensional representation of the interaction between the centrifugation speed (rpm) and the centrifugation time (min) impacts on both cell integrity and recovery efficiency of *D. salina* cells.

We notice that cell integrity decreased gradually once the centrifugation speed and centrifugation time exceeded 6500 rpm and 10 minutes, respectively. The highest cell integrity percentage (97%) was registered at 5000 rpm and 5 minutes, respectively. Conversely, increasing the centrifugation speed along with increasing the centrifugation time influences positively the recovery efficiency. Hence, the maximum response achieved is 99% when centrifugation speed and time variables are at their highest tested level 8000 rpm and 15 minutes, respectively.

The effects of centrifugation speed and salinity on the cell integrity and recovery efficiency of *D. salina* were depicted in Figure 6c and 6d, respectively. Centrifugation speed strongly affects cell integrity and recovery efficiency. Indeed, beyond 6500 rpm, cellular integrity decreases showing an antagonistic behaviour in comparison with recovery efficiency which notably increases. Salinity did not exhibit any impact on cellular integrity within the tested concentration range. Conversely, we observed that at higher concentrations, salinity negatively impacted recovery efficiency. The estimated quadratic coefficients for centrifugation speed were statistically significant for both responses, while the linear coefficient X_2 related to salinity concentration was not statistically significant for cell integrity confirming this result.

The interaction between time and salinity variables, as well as their impact on cellular integrity and recovery efficiency are illustrated in Figures 6e and 6f. We note the absence of any interaction effect between the two indicated factors on cell integrity. However, the significant influence of the time variable was clearly revealed, unlike salinity, which showed no impact. Nevertheless, both indicated factors impact significantly the recovery efficiency, as demonstrated by the calculation of their quadratic coefficients (Table 3).

Based on this analysis, we can conclude that the optimal separation conditions for *D. salina* regarding cell integrity and recovery efficiency are as follows: centrifugation speed of 6500 rpm, extraction time of 10 minutes, and salinity of 70 g/L.

Beyond these values, higher centrifugation speed and duration negatively impact cell

Table 3. ANOVA for the effect of speed, salinity and time on cell integrity and recovery efficiency using the quadratic response surface model

Source	Cell integrity (%)					Recovery efficiency (%)			
	Df	Sum of squares	Mean square	F value	P value	Sum of squares	Mean square	F value	P value
Model	9	6022.24	2.65	18.55	0.0021	6642.47	3.77857	26.45	0.0003
X_1	1	3741.13	3741.13	1411.75	0	4095.13	4095.13	1083.78	0.0000
X_3	1	364.5	364.5	137.55	0	378.125	378.125	100.07	0.0000
X_2	1	0.125	0.125	0.05	0.8343	98	98	25.94	0.0014
X_1^2	1	1730.84	1730.84	653.15	0	1613.39	1613.39	426.98	0.0000
X_1X_2	1	4	4	1.51	0.2589	6.25	6.25	1.65	0.2393
X_1X_3	1	0.25	0.25	0.09	0.7677	4,00	4	1.06	0.3378
X_2^2	1	86.2132	86.2132	32.53	0.0007	210.761	210.761	55.78	0.0001
X_2X_3	1	1	1	0.38	0.5584	1	1	0.26	0.6228
X_3^2	1	6.84474	6.84474	2.58	0.1521	78.7605	78.7605	20.84	0.0026

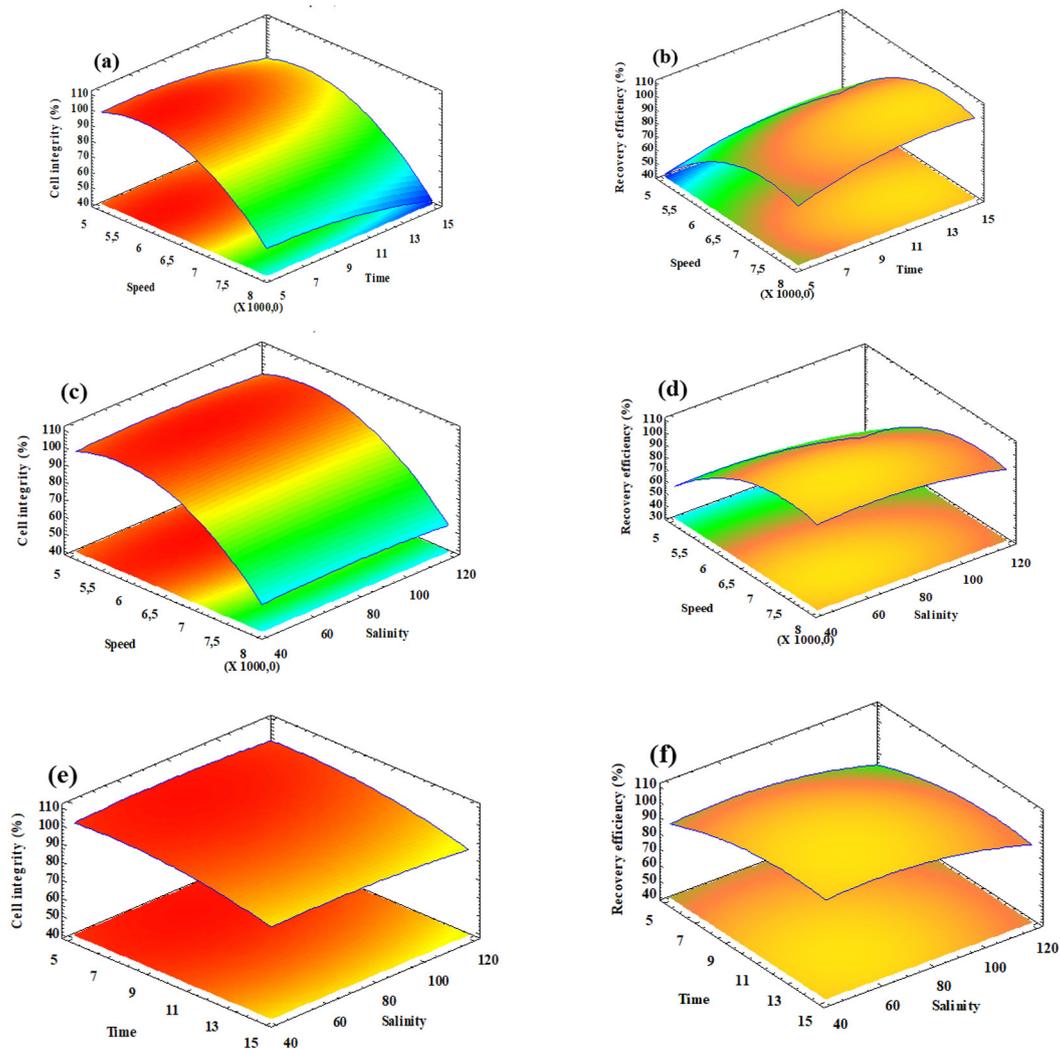


Figure 6. Spatial representation and response surface plots showing the interactive effects of centrifugation speed (X_1), salinity (X_2) and separation time (X_3) on the cell integrity and recovery efficiency of *D. salina*

integrity, while increased salinity reduces recovery efficiency. Conversely, lower centrifugation speed and duration also decrease recovery efficiency. The three variables tested independently influence both recovery efficiency and the cell integrity of *D. salina* cells.

Experimental validation of the model

To evaluate the predictive accuracy of the model, all tests were validated experimentally. Results indicate that, following dilution, centrifugation effectively separates the microalgal biomass. To confirm this observation, the carotenoid content in the paste was measured. Overall, carotenoid levels were influenced by the three parameters tested, with centrifugation speed and salinity having the most significant effects ($p < 0.05$) (Table 3).

The highest carotenoid yields were observed at centrifugation speeds below 6500 rpm and at a

moderate salinity of 72.5 g/L, with values ranging from 4.33 to 4.94% (w/Fw). In contrast, at 6500 rpm and a higher salinity of 105 g/L, the carotenoid content decreased to 3.16%, which may be explained by the presence of residual salts in the biomass that negatively affect carotenoid recovery (Table 4). These findings underline the importance of introducing a dilution step to remove residual salts, thereby improving both carotenoid yield and the market value of the obtained paste. Table 4 further supports these observations by showing the presence of carotenoids in the supernatant obtained at 8000 rpm. An increase in carotenoid levels was also detected with longer centrifugation times, suggesting progressive cell disruption. This interpretation is consistent with the results of cell integrity, which are in agreement with the predicted values summarized in Table 2.

Table 4. Cell integrity of recovered *D. salina* paste and UV spectrophotometry in the supernatant after centrifugation

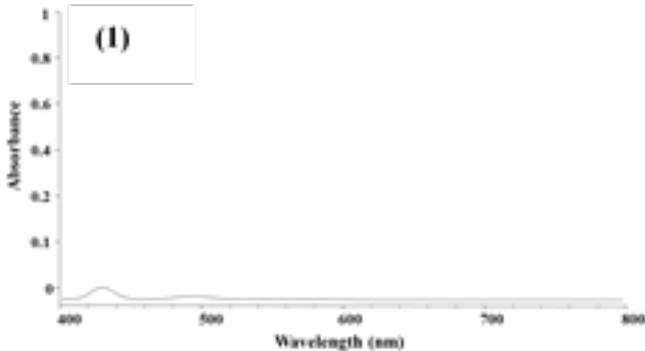
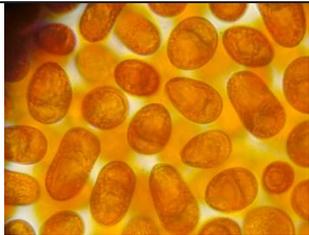
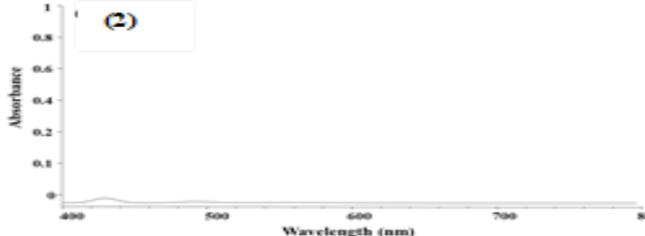
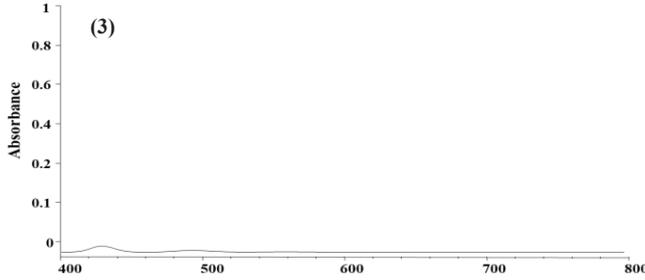
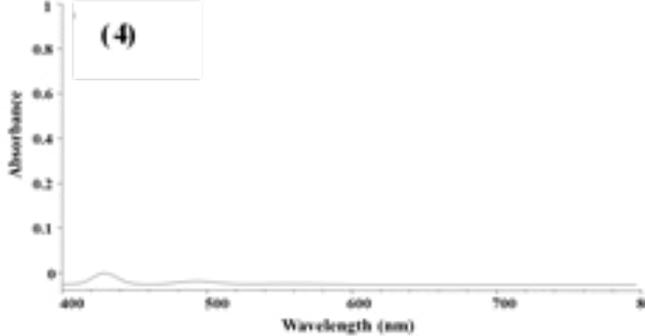
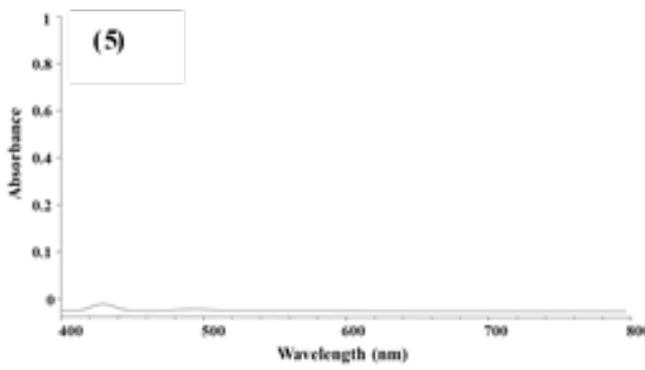
No.	Figures	UV spectrophotometer	Presence/absence of carotenoids
1			Absence
2			Absence
3			Absence
4			Absence
5			Absence

Table 4. Cont. Cell integrity of recovered *D. salina* paste and UV spectrophotometry in the supernatant after centrifugation

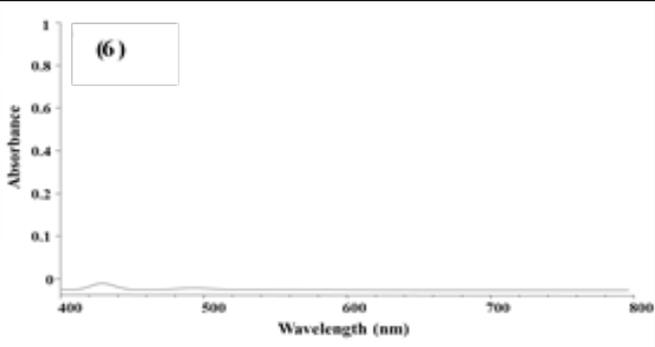
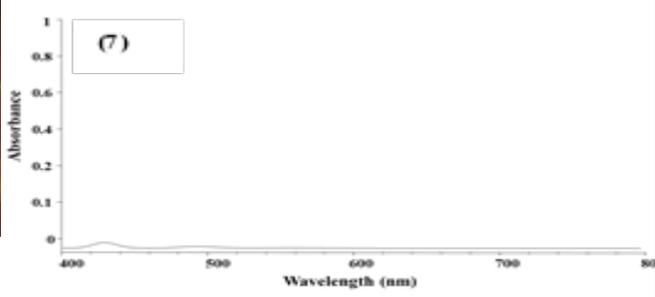
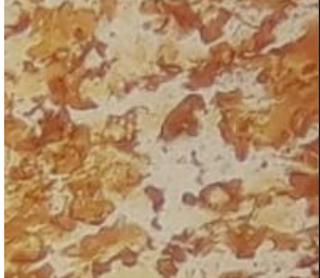
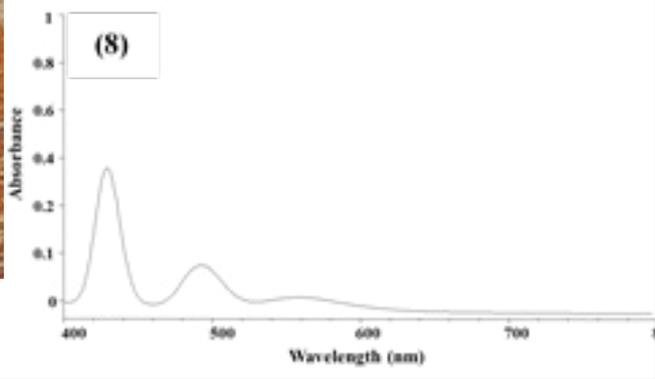
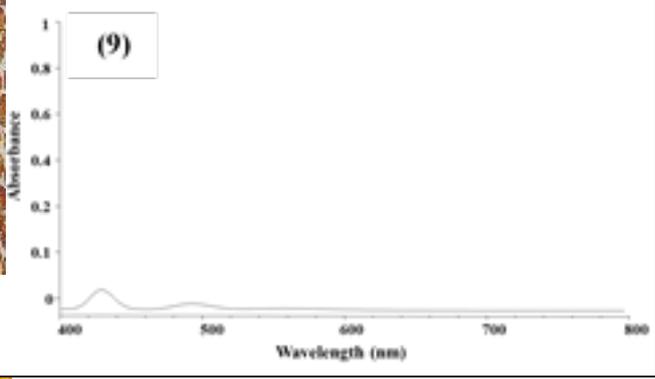
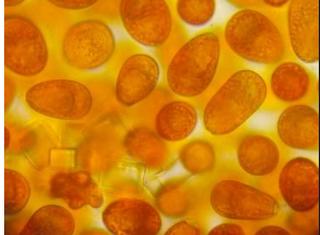
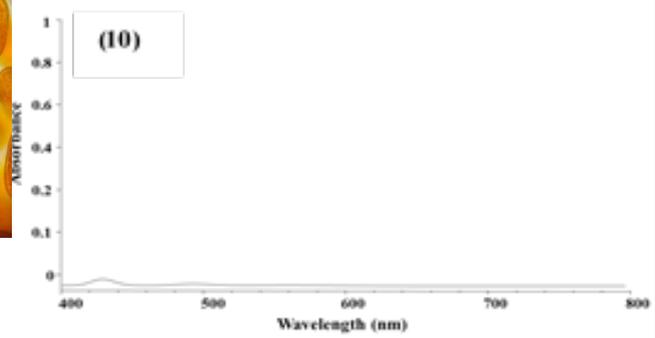
6			Absence
7			Absence
8			Presence
9			Absence
10			Absence

Table 4. Cont. Cell integrity of recovered *D. salina* paste and UV spectrophotometry in the supernatant after centrifugation

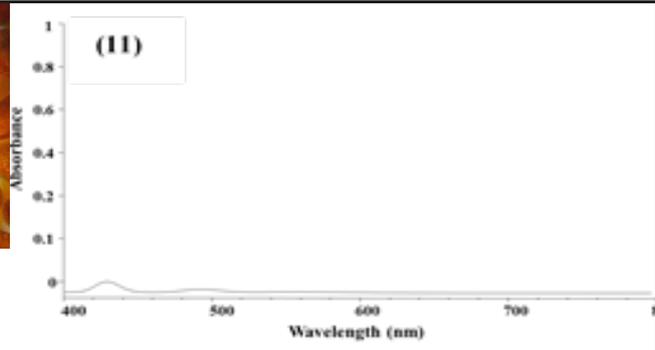
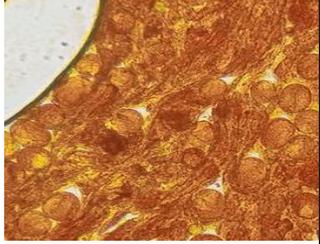
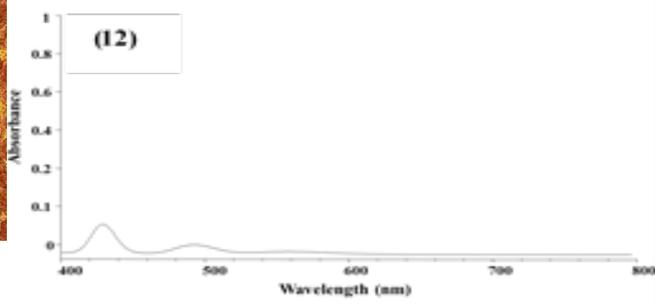
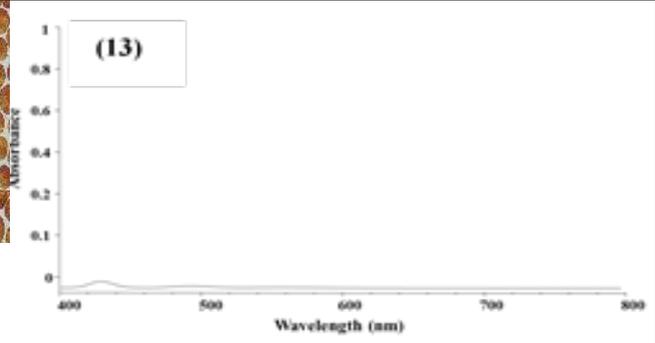
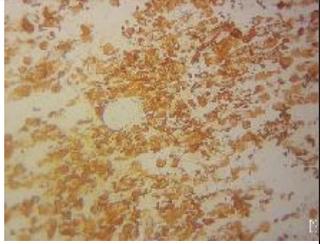
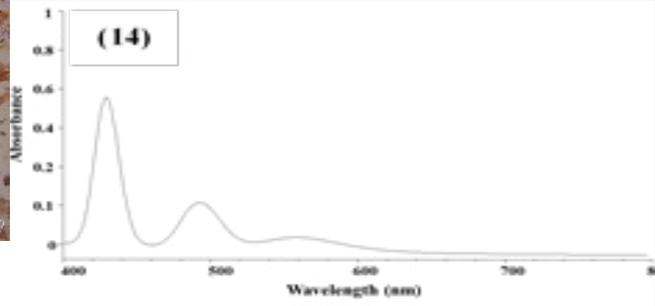
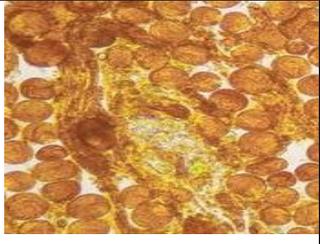
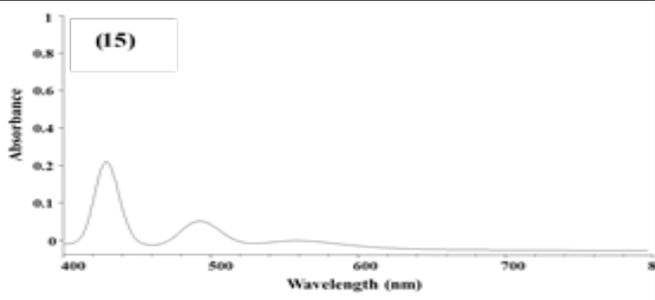
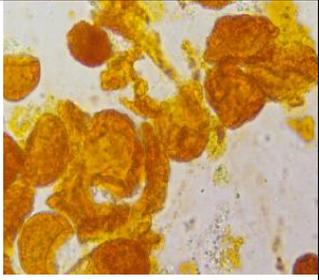
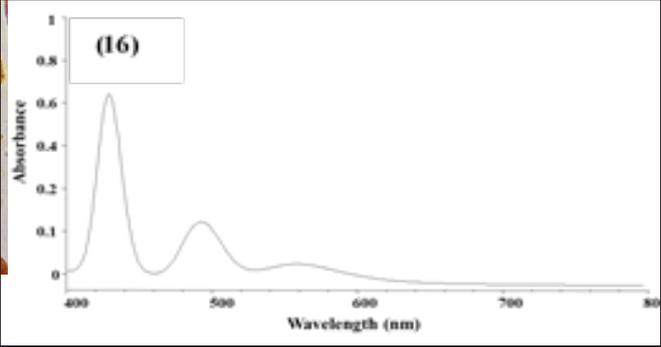
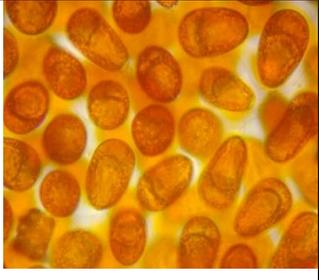
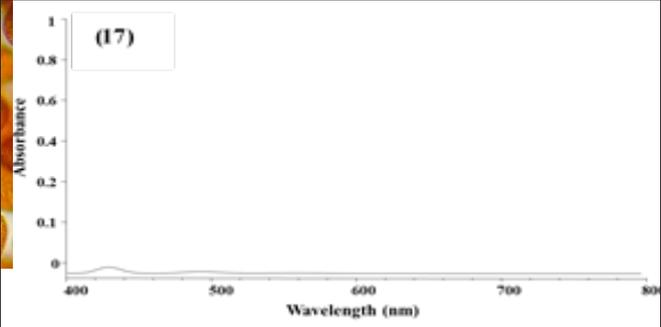
11		 <p>(11)</p>	Absence
12		 <p>(12)</p>	Absence
13		 <p>(13)</p>	Absence
14		 <p>(14)</p>	Presence
15		 <p>(15)</p>	Presence

Table 4. Cont. Cell integrity of recovered *D. salina* paste and UV spectrophotometry in the supernatant after centrifugation

16			Presence
17			Absence

Moreover, the decrease in carotenoid content observed under prolonged centrifugation conditions can also be attributed to excessive cell lysis. This conclusion is confirmed by the cell integrity analysis and remains consistent with the predicted values reported in Table 5.

Comparing the experimental values for recovery efficiency and cell integrity with the model's predictions, we find that under the optimal detected conditions, a centrifugation speed of 6500 rpm, salinity of 70 g/L, and centrifugation time of 10 minutes, the values achieved were 95.79% for cell integrity and 98.79% for recovery efficiency. These experimental results closely match the predicted values of 97% for cell integrity and 96% for recovery efficiency, demonstrating the model's accuracy.

Our results align with findings from previous studies conducted by Heasman et al., (2000) and Chen and Jiang (2009) who investigated microalgae centrifugation and demonstrated high recovery efficiency with minimal cell damage over short periods. Similarly, Xu et al., (2015) conducted a laboratory-scale study on *D. salina* for biodiesel production, subjecting it to various centrifugal forces ranging from 1000 to 15000 rpm for 10 minutes. They found that cell destruction occurred when the centrifugal force exceeded 5000 rpm, resulting in approximately 40% loss of

glycerol yields and cell integrity, at forces greater than 9000 rpm. Additionally, Yaich-Achouri et al., (2021) reported that the recovery efficiency of *D. salina* DZI is highest at 2500 rpm with a centrifugation time of 10 minutes. They also demonstrated that this method is more effective than flocculation in preserving the yield and composition of *D. salina* fatty acids, which are a valuable source of biodiesel.

These investigations confirm the effectiveness of the tested centrifugation method for recovering *D. salina*. Our findings further reveal that *D. salina* from Sabkhet Sidi El Heni shows a relative resilience to centrifugal forces when compared to the previous studies, achieving recovery efficiency and cell integrity rates both above 95% at 6500 rpm and 10 minutes centrifugation time. Notably, the previous studies cited above, have not explored biomass washing, a step critical for reducing salt content, which in turn raises carotenoid levels and boosts biomass value. Unlike prior work that optimized centrifugation without preconcentration, this study employs a natural flotation preconcentration phase, followed by a washing and dewatering sequence. This approach effectively lowers salt content while increasing carotenoid yield, significantly enhancing the biomass quality and market value.

Table 5. Total carotenoids content in the recovered paste

Run	Speed (rpm)	Salinity (g/l)	Time (min)	% (w/Fw) carotenoids in the paste
1	6500	72.5	10	4.79 ± 0.03 ^a
2	6500	72.5	10	4.86 ± 0.05 ^a
3	6500	72.5	10	4.94 ± 0.04 ^a
4	6500	72.5	10	4.78 ± 0.06 ^a
5	6500	72.5	10	4.80 ± 0.08 ^a
6	5000	72.5	15	4.33 ± 0.07 ^b
7	5000	72.5	5	4.90 ± 0.02 ^a
8	8000	105	10	1.19 ± 0.05 ^j
9	6500	105	15	2.56 ± 0.07 ^e
10	6500	105	5	3.08 ± 0.11 ^d
11	6500	40	5	4.81 ± 0.02 ^a
12	6500	40	15	3.7 ± 0.03 ^c
13	5000	105	10	3.16 ± 0.03 ^d
14	8000	40	10	2.13 ± 0.02 ^g
15	8000	72.5	5	2.33 ± 0.04 ^f
16	8000	72.5	15	1.85 ± 0.05 ^h
17	5000	40	10	4.83 ± 0.02 ^a

Note: The results are expressed as ± SD, in triplicated experiments. Statistical analysis was performed using ANOVA followed by Tykey’s test. Values followed by different letters are significantly different at the limit of p<0.001.

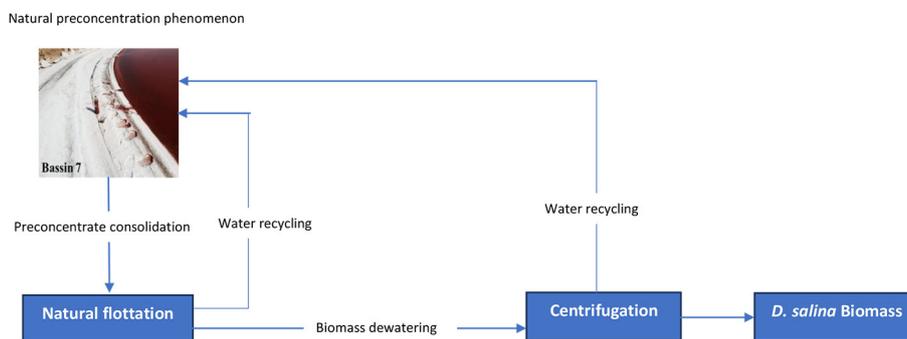


Figure 7. *D. salina* harvesting-dewatering process in Sabkhet Sidi El Heni saltworks

CONCLUSIONS

Harvesting and dewatering *D. salina* biomass is a crucial step for its exploitation, especially in high-salinity environments like Sabkhet Sidi el Heni. The extreme conditions and wind in these salt lagoons naturally concentrate *D. salina* along the basin edges. This preconcentration phenomenon is facilitated by the high brine density together with the hydrophobic nature of *D. salina*, rich in carotenoids and lipids, allowing for flotation and creating an initial concentration step advantageous for harvesting. Direct centrifugation of *D. salina* suspension using a laboratory centrifuge was ineffective for biomass separation

due to the high brine density and the low density of the microalga. On the other hand, laboratory flotation tests proved effective, demonstrating a concentration factor of 10.71 and a recovery efficiency of 37%. Although effective as a secondary concentration stage, flotation alone is insufficient for complete dewatering, necessitating a final centrifugation step. The combined approach of flotation followed by centrifugation yields positive results at laboratory scale, enabling cost and energy reduction. However, to facilitate cell decantation, the centrifugation stage requires prior dilution to lower salt levels in the preconcentrate. The dilution also serves as a washing step, reducing salt content and enhancing product quality. Optimized

centrifugation parameters at laboratory scale, determined through a Box-Behnken Design, established the ideal conditions (70 g/L salinity, 6500 rpm, 10 minutes), achieving a recovery efficiency and cell integrity of 95.79% and 98.79%, respectively, consistent with model predictions.

The three-phase process, natural preconcentration at the pond edges, flotation, and centrifugation, offers a cost-effective, energy-efficient approach well-suited for saline environments. Furthermore, these results indicate that the dilution step is essential for removing excess salt from the biomass, which increases carotenoid content and thereby enhances its market value. The water removed through natural flotation, along with that used in the centrifugation process, can be directly recycled back into the salt basins, as it remains free of any chemical additives (Figure 7).

Future work should focus on (1) optimizing flotation to maximize recovery without chemicals that could impact both salt and *D. salina* biomass quality and (2) selecting appropriate dewatering equipment for optimal cost and energy efficiency well suited with the physicochemical characteristics of *D. salina* suspension of salt pans.

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