

Scale-up of spirulina protein extraction process for pilot reactor design

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Abstract

This investigation focuses on designing a 400 L pilot reactor for extracting proteins from *Spirulina* using the method of scale-up dimensionless numbers. The first step was to determine the factors to study the extraction at 0.5 L, such as temperature, time, and agitation. Then, by using statistical methods, we identified the optimal extraction yield point. Subsequently, at the 4 L scale, we tested different impellers to find specific dimensionless number ratios, which were useful in designing the 400 L reactor, in addition to estimating stirring speeds for maximum protein extraction. The next step in this process is the isolation of protein.

Keywords: agitators, dimensionless numbers, protein, reactor, scale-up, spirulina

Introduction

The rapid growth of the global population, projected to exceed 8.5 billion by 2030, poses a critical challenge to food security (Arora & Mishra, 2024). Conventional protein sources, such as meat and dairy, require large amounts of land, water, and energy, contributing to environmental degradation and greenhouse gas emissions (Kumar *et al.*, 2022). Additionally, climate change and resource limitations further threaten agricultural productivity, making it increasingly difficult to meet the rising demand for high-quality protein (Kusmayadi *et al.*, 2021). Protein consumption in people’s daily diets is essential for building muscle, as well as for body composition and the activity of the immune system (Ferrari *et al.*, 2022). Given these challenges, the search for alternative protein sources has become essential to ensuring a sustainable and nutritionally adequate food supply. Protein plays a crucial role

in human health, supporting muscle development, body composition, and immune function (Ferrari *et al.*, 2022).

Among the promising alternatives, the cyanobacterium *Arthrospira platensis*, commonly known as spirulina, stands out as a sustainable source of various compounds of interest, such as proteins, vitamins, and trace elements (Thevarajah *et al.*, 2022). *Spirulina* has been explored for various applications, including the extraction of phycocyanin—a pigment-protein complex valued for its anti-inflammatory and neuroprotective properties in medicine and as a natural blue dye in the food industry (Tavanandi *et al.*, 2018). Additionally, lysozyme derived from *spirulina* has been studied for its potential use in antimicrobial food packaging (Benelhadj *et al.*, 2016). With a protein content ranging from 55% to 70%, along with essential amino acids, *spirulina* represents a valuable source of alternative protein (Marzorati *et al.*, 2020).

Despite its high protein content, the industrial utilization of microalgae proteins has faced challenges due to the presence of non-protein components such as chlorophyll, which can cause undesirable changes in color, taste, and texture (Becker, 2007). Over time, interest in industrial *spirulina* production has increased, involving processes such as cultivation, concentration, and drying (Richmond, 2003). A critical step in protein extraction is the disruption of the cell membrane, which can be achieved through mechanical, chemical, or energy-based methods (Rausch, 1981). Mechanical techniques, such as high-pressure homogenization, apply forces up to 2000 atm to break cells (de Boer *et al.*, 2012). Chemical methods involve alkaline hydrolysis with NaOH or KOH at pH 9–11, which is effective for protein extraction but unsuitable for lipid recovery due to the risk of saponification (Flores Ramos *et al.*, 2017). Energy-based methods, such as ultrasound, use cavitation at frequencies of 10–20 kHz to disrupt cell membranes (Safi *et al.*, 2014). Among these approaches, alkaline extraction is particularly suitable for microalgae protein extraction.

Scaling up protein extraction processes requires a thorough understanding of critical performance phenomena, such as reaction kinetics, heat distribution, mixing, and residence time (Bonem, 2018; Makkawi, 2014). These factors influence process efficiency and can lead to unexpected outcomes with economic consequences (Reisman, 1993). A key approach to process scale-up is dimensional analysis, which defines relevant dimensionless numbers to maintain process similarity between laboratory and industrial scales (Harmsen, 2019).

This research focuses on the extraction of proteins from *spirulina* using dimensionless numbers, which are essential in engineering design as they provide insights into mass, heat, and momentum transport phenomena (Otálvaro-Marín & Machuca-Martínez, 2020). A key distinction of this study is the use of up to 120 g of *spirulina* for extraction, whereas most previous studies report extractions using only 1 g or less. Scale-up was achieved by maintaining energy distribution and shear forces using dimensionless parameters such as the Reynolds number (Re) and the power number (Np) (Başbuğ *et al.*, 2017; Kaiser *et al.*, 2017). Experiments were conducted at different stirring speeds to establish a correlation between Re and Np, facilitating the transition to a larger reactor while preserving protein extraction efficiency at an industrial level (Xie *et al.*, 2022).

The choice of agitators plays a crucial role in reactor performance. Options include large-diameter impellers (70–90% of reactor diameter) and small-diameter impellers (25–50%), which generate axial or radial flows. Hydrofoil impellers are particularly effective for producing axial flow with minimal shear effects (John *et al.*,

2023; Pericleous & Patel, 1987). Additionally, computational fluid dynamics (CFD) simulations, based on the Navier-Stokes equations, were employed to predict flow behavior by applying mass balance, momentum conservation, and energy conservation principles (Harris *et al.*, 1996).

This study aims to design a 400-liter pilot reactor for protein extraction from *spirulina* using the dimensionless number approach. The process was evaluated in a 0.5 L laboratory reactor, followed by validation in a 4 L reactor to ensure scalability.

Materials and Methods

Materials

Impeller and baffles (stainless steel material of our design and manufacture) for beakers type Pyrex® of 0.5 and 4.2 L capacity. UV-Vis Spectrophotometer Thermo Fisher® Model Genesys 150, vertical stirrer EuroTech® 370, centrifuge Thermo Fisher® Model SOVALL ST 16R, water bath Brand TOMOS® of 20 L, manual pH meter Hanna® HI98103 with HI 1271 electrode, amperometric clamp Brand PRASEK® mod PR-202. Folin-Ciocalteu reagent Sigma Aldrich, *Spirulina* Powder from Qingdao Huineng Biotech Co., Ltd., copper sulfate pentahydrate, sodium carbonate, sodium potassium tartrate tetrahydrate, potassic soda pearl, and caustic soda pearls.

Optimal reaction parameters at 0.5 L scale

The baffles were designed for a 600 mL Pyrex® brand beaker as a reactor for 0.5 L tests, with the following measurements: T = 8.6 cm inner diameter, Z = 8.6 cm liquid height, B = T / 12 = 73 millimeters baffle width, and D = 3.8 cm factory impeller diameter. The factors to be considered were: pH, temperature, amount of *spirulina* per unit volume, stirring speed, impeller type, time, and the dimensions of the laboratory reactor. Only mechanical stirring was applied to the sample. An experimental design was carried out according to Table 1 with a first-order 2k model, including a central point with 4

Table 1. Factors and levels considered in the design of experiments.

| Variables for the design of experiments | | Level code | | |
|---|-----|------------|------|-----|
| | | -1 | 0 | 1 |
| Time | min | 30 | 50 | 70 |
| Impeller speed | rpm | 100 | 500 | 900 |
| Temperature | °C | 20 | 32.5 | 45 |

repetitions, in which the linear influence of each factor was evaluated. A second-order model was then applied to find the optimal points using the Box-Behnken model. The concentration of *spirulina* subjected to the alkaline hydrolysis process was 30 g L⁻¹. The operating conditions in the first test were a glass volume of 500 mL, pH between 9.5 and 10, RPS of 15, room temperature (21 °C), and sampling times of 15, 30, 50, 65, and 85 minutes. The operating conditions in the second test were similar to the above, with only a difference in the RPS (revolutions per second) of 1.67 and sampling times of 11, 20, 63, 78, and 95 min.

Optimal reaction parameters at 4.0 L scale

The tests for impeller selection were carried out in a 4 L reactor, in which the Pitched 45°, 3-fin marine blade, Hydrofoil, and Rushton models were designed. The vessel used was a 4.5 L Pyrex® brand beaker ($Z = 22$ cm, $T = 15.2$ cm, $Z/T = 1.45$) with spacings from the base and the impellers ($C = T/4 = 3.8$ cm, $S = 2Z/3 - T/4 = 11.7$ cm) and an impeller diameter of 6 cm.

The order of incorporation was as follows: water at 45 °C in a water bath was added first, then *spirulina* (120 g) was introduced, followed by the addition of caustic potash to obtain a pH between 9.5 and 10. Finally, each impeller to be evaluated was added. The power of the agitator was determined using an amperometric clamp.

Protein quantification

The evaluation of the reactor efficiency at its various stages was assessed by the spectrophotometric determination of the protein content following the modified Lowry method, for which bovine serum albumin was used as a standard in a concentration range of 0–100 mg L⁻¹ (Ramírez-Rodriguez *et al.*, 2021).

After alkaline hydrolysis, a sample of approximately 50 g was taken and centrifuged at 20 °C in a Thermo Scientific Sorvall ST 16R model for 10 minutes at 3500 g. Then, 100 µL of supernatant was diluted 20 times to ensure that the protein concentration was within the calibration curve. Next, 700 µL of Lowry's solution was added to 500 µL of this dilution, and the resulting solution was shaken for 20 seconds. The sample was then incubated for 20 minutes in the dark. After this time, 100 µL of Folin reagent was added and immediately mixed using a vortex mixer. The resulting solution was incubated for 30 minutes at room temperature in the dark. The resulting blue solutions were measured at a wavelength of 750 nm on a Thermo Fisher Genesys 150 UV spectrophotometer (Dulekgurgen, 2005).

Scaling up the reactor from 4 L to 400 L

Power prediction

Based on the data obtained from the electrical consumption for the 4 L reactor and maintaining geometric similarity to design a 400 L reactor, the prediction of the amount of energy needed was made by considering the energy used in the experimental tests, using the kW/m³ values shown in Table 2.

Determination of the rotation speed range

The operating rotation of the 400-liter reactor will be determined using the Np versus Re graph obtained in the experimental part. The Np data will be evaluated based on the Re values for the 400 L reactor diameter.

CFD simulation for 400 L reactor

The simulation for the extraction of proteins using different impellers was performed within the COMSOL Multiphysics 6.0 software application in the mixing module, using the rotational speed in RPS indicated in "Table 3" for three of the agitators under evaluation (Pitched, Hydrofoil and Rushton).

Table 2. Typical power per unit volume values in industry (Towler & Sinnott, 2021).

| Type of agitation | Applications | kW/m ³ |
|-------------------|---------------------------|-------------------|
| Mild | Simple mix | 0.04–0.1 |
| | Homogeneous reactions | 0.01–0.03 |
| Half | Heat transfer | 0.03–1.0 |
| | Liquid – liquid mixture | 1.0–1.5 |
| Severe | Suspension of solids | 1.5–2 |
| | Gas absorption | 1.5–2 |
| | Emulsions | 1.5–2 |
| Violent | Suspension of fine solids | > 2 |

Table 3. Rotational speed in RPS.

| Marine | Hydrofoil | Pitched | Rushton |
|--------|-----------|---------|---------|
| 4.3 | 5 | 4.8 | 2.8 |

Table 4. Reactor dimensions maintaining the Z/T ratio of 1.45.

| Scenario 1 | Amount | Scenario 2 | Amount |
|------------|--------|------------|--------|
| V 1 (L) | 4 | V 2 (L) | 400 |
| Z 1 (cm) | 22 | Z 2 (cm) | 102.2 |
| T 1 (cm) | 15.2 | T 2 (cm) | 70.6 |
| D 1 (cm) | 6 | D 2 (cm) | 28 |

V = volume, T = inner diameter, Z = liquid height, D = impeller diameter.

Boundary conditions on the reactor's walls were set to no-slip boundary conditions. The boundary conditions on the rotating impellers were set to rotate with the same velocity as the no-slip counterclockwise rotation conditions. Two separate domains were then created: one for fixed parts such as walls and baffles, and the other for rotating parts. These domains were non-overlapping. Next, the domains were linked, and the rotating domain was defined in the simulation software. A normal mesh was selected, and the application was executed. This experimental investigation was conducted on a 4-liter scale, providing sufficient data to propose a design for a 400-liter pilot reactor.

Pilot reactor design

Based on the data obtained in the previous stages, the dimensions of the reactor, impeller, construction material, reactor heating, and multiparameter monitoring instrumentation will be determined.

Statistical analysis

An experimental design was applied using a first-order 2k model with a central point and 4 repetitions. The ANOVA test was applied at a confidence level of 95%. A second-order model was then applied to find the optimal points using the Box-Behnken optimization design. The statistical package used was Minitab v.19.

Results and Discussion

Table 5 presents the results of the exploratory tests conducted at a 0.5-liter scale, with time and stirring speed as the study factors. The results indicate that extraction performance shows no significant variation beyond 60 minutes, and agitation has no substantial effect. Based on

these findings, the study range for optimizing extraction performance was set between 30 and 70 minutes and from 100 to 900 RPM. Regarding temperature, the guidelines proposed by Lupatini *et al.* (2017) were applied, and a temperature below 60 °C was used to avoid protein denaturation, with a maximum of 45 °C selected.

Other factors, such as the optimum pH of 9.5 based on Benelhadj *et al.* (2016), the spirulina water mass ratio of 30 g/L, and the maximum temperature of 60 °C were taken from Lupatini *et al.* (2017) research. A maximum temperature of 45 °C was selected for this research because the interaction with time and agitation can improve the extraction without unnecessarily raising the temperature, thereby reducing energy costs and the operation time of the batch-type pilot reactor.

In the presence of curvature, the ANOVA test shows a p-value of 0.008 (< 0.05), so we proceed with a second-order search using the Box-Behnken design. It is verified that temperature has a significant influence, both as a linear term and quadratic, as well as in interaction with time, but stirring speed does not. The terms are

Table 5. Preliminary results of laboratory-scale testing.

| Test - 100 rpm | | Test - 900 rpm | |
|----------------|------------------------|----------------|------------------------|
| T (min) | Extraction performance | T (min) | Extraction performance |
| 0 | 23.1 % | 0 | 23.1 % |
| 20 | 31.3 % | 15 | 31.9 % |
| 40 | 38.0 % | 30 | 40.7 % |
| 63 | 41.9 % | 50 | 48.1 % |
| 78 | 47.9 % | 60 | 50.3 % |
| 95 | 50.9 % | 85 | 50.2 % |

Table 6. ANOVA.

| Source | GL | SC Ajust. | RMSE | Valor F | Valor p |
|-------------------------------------|----|-----------|---------|---------|---------|
| Model | 5 | 365.063 | 73.013 | 53.82 | 0.000 |
| Lineal | 2 | 265.331 | 132.666 | 97.79 | 0.000 |
| Temperature (°C) | 1 | 256.511 | 256.511 | 189.07 | 0.000 |
| Time (Min) | 1 | 8.820 | 8.820 | 6.50 | 0.029 |
| Square | 2 | 86.041 | 43.021 | 31.71 | 0.000 |
| Temperature (°C) * Temperature (°C) | 1 | 66.016 | 66.016 | 48.66 | 0.000 |
| Time (Min) * Time (Min) | 1 | 20.026 | 20.026 | 14.76 | 0.003 |
| 2-factor interaction | 1 | 13.690 | 13.690 | 10.09 | 0.010 |
| Temperature (°C) * Time (Min) | 1 | 13.690 | 13.690 | 10.09 | 0.010 |
| Error | 10 | 13.567 | 1.357 | | |
| Total | 15 | 378.629 | | | |

significant in terms of linear, quadratic, and interaction effects. The adjustment for correlation was made, and we obtained an R^2 of 96.43%. A contour graph was also obtained (Figure 2), where the maximum yield is evident in the center of the area indicated in dark green, where the highest yield of 48% is reached approximately at 48 minutes and 42 °C.

The equation resulting from the correlation between the variables time and temperature was the following:

$$\begin{aligned} \text{Performance (\%)} = & \\ & -24.38 + 2.513 T(^{\circ}\text{C}) + 0.852 \text{ Time (Min)} \\ & - 0.02600 T^2 (^{\circ}\text{C}) - 0.00559 \text{ Time}^2 (\text{Min}) \\ & - 0.00740 T(^{\circ}\text{C}) * \text{Time}(\text{Min}) \end{aligned}$$

According to the response surface diagram, the optimum conditions for achieving a Spirulina protein yield of 48.42% are reached when a temperature of 41.46 °C and a time of 48.59 minutes are applied.

With the optimal conditions obtained at the 0.5-liter scale, the 4-liter stage followed. In this stage, we have a larger volume that allows us to evaluate the performance of the impellers (Paul *et al.*, 2004). In this study, we evaluated both radial and axial-type impellers. The axial-type impellers include the marine, hydrofoil, and pitched types, while the radial-type impeller is the Rushton.

Table 7. Model summary.

| S | R-cuad. | R-cuad.(adjusted) | R-cuad.(pred) |
|---------|---------|-------------------|---------------|
| 1.16477 | 96.42% | 94.63% | 82.46% |

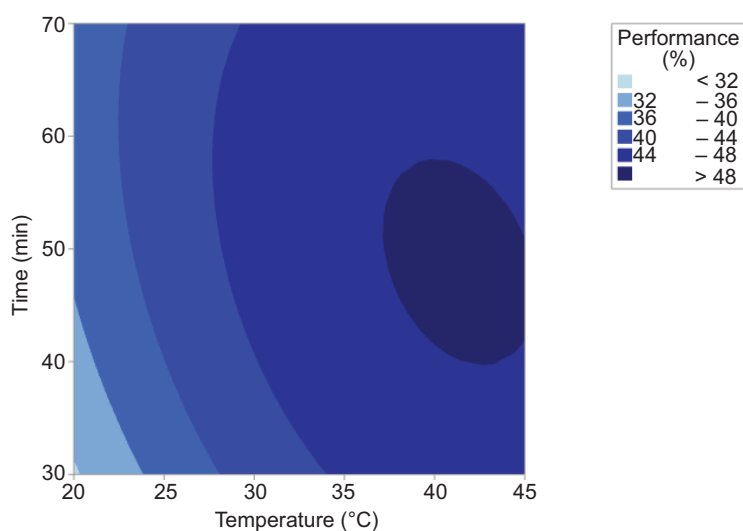


Figure 1. Contour plot showing spirulina yield as a function of temperature and time.

The experiments at the 4-liter scale were carried out under optimal temperature conditions, with an agitation speed of 500 RPM. Samples were taken up to minute 75.

The results are shown in Figure 3. We can see that the performance of the axial agitators is very similar; however, the radial impeller stands out, achieving almost the maximum extraction in half the time of the experiment.

The prediction of the power number involves the relationship between $\text{Log}(N_p)$ and $\text{Log}(Re)$. In Figure 4, we also verify that the Rushton impeller demands more energy than the other three impellers.

The correct way to interpret Figure 5 is to understand that it corresponds exclusively to the 4-liter reactor and the impellers used in this investigation (Ma, 2014). We will be able to predict the energy required for a larger-scale reactor if we maintain geometric similarity, meaning that the same geometric relationships are upheld (Tattersson, 2002) in the dimensions of the vessel, the baffles, and the impeller used in this experiment (McCabe *et al.*, 2004).

Figure 4 was used to determine the required power. This energy requirement, linked to the rotational speed of the 400 L pilot reactor, must meet the criteria for maintaining a constant P/V ratio and the same degree of turbulence. These criteria were achieved with rotational speeds in RPS of 4.3 (Marine), 5 (Hydrofoil), 4.8 (Pitched), and 2.8 (Rushton), with powers ranging from 590 to 630 watts.

After completing the 4-liter stage, a CFD analysis was performed on a scaled pilot reactor to predict its hydrodynamics. The shear rate graphs for each impeller were simulated (Figure 5) for both axial and radial impellers,

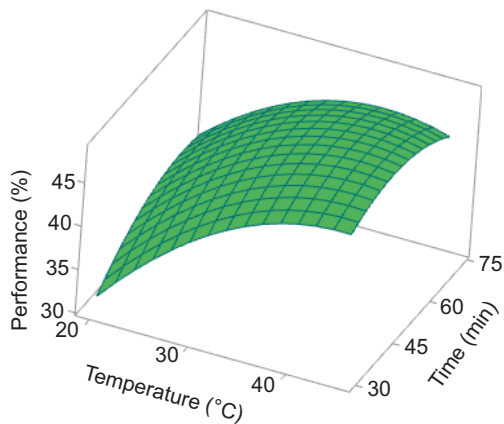


Figure 2. Response surface diagram for optimizing spirulina yield in relation to temperature and time.

except for the marine impeller, which was not available in the CFD subprogram of the COMSOL Multiphysics software. The shear rate graphs for the axial impellers, such as the Hydrofoil and Pitched 45° impellers, show very similar patterns in the areas close to the impellers. These graphs indicate a small area with a considerable velocity gradient where the shear rate is higher, with only slight differences in intensity between the Hydrofoil and Pitched 45° impellers. Therefore, the performance of these impellers was considered equivalent. In the Rushton impeller, this high shear rate area is much larger, even with a lower shaft rotation speed of 2.8 RPS, compared to the Hydrofoil at 5 RPS and the Pitched at 4.8 RPS.

These results were significant because they validated the higher performance of the Rushton impeller compared to the radial impellers in the 4-liter stage.

Finally, regarding the design of the 400 L pilot reactor, it is estimated that for optimal performance, the reactor should have the main characteristics as projected in Table 4. It should have a diameter of 70.6 cm, a height of 102.2 cm with an additional 28 cm due to foam generation. The internal diameter should be 72 cm, while the selected Rushton-type impeller should have a disk diameter of 21 cm, a blade length of 10 cm, a blade width of 6 cm, and a blade-to-disk adhesion length of 3 cm. The construction material should be food-grade 316 stainless steel, resistant to NaOH corrosion. The operating parameters imply 45 minutes of extraction at a temperature of 45 °C and 168 RPM.

Conclusions

The critical variables and their values to achieve optimal extraction are a temperature of 45 °C, a time of 48 minutes, a pH range of 9.5–10, and a spirulina/water mass ratio of 30 g/L. For the 4-liter laboratory reactor, we found that using a Rushton-type impeller achieves the maximum yield of approximately 50% in the shortest possible time of 30 minutes. This is because this type of impeller generates shear forces that improve the breaking of the spirulina cell wall. Using the dimensionless number scaling method, it was possible to design the 400-liter pilot reactor, which allows us to extract proteins with a high probability of achieving maximum yield. The dimensions of the reactor would be 70.6 cm in diameter and 102.2 cm in height, and the impeller should be a Rushton type with a diameter of 28 cm and a 21 cm disk.

This research outlines a method for designing and operating an industrial reactor at the pilot level, beginning with an understanding of the most significant factors in

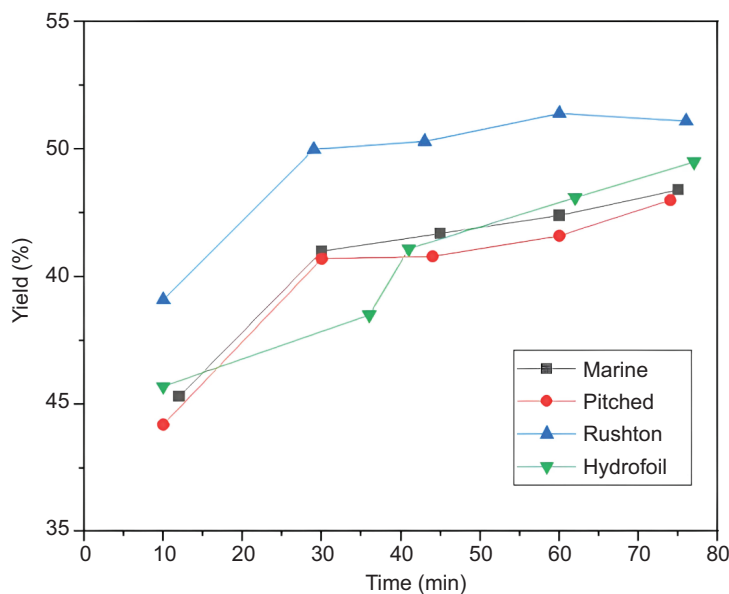


Figure 3. Performance of each impeller over time for the 4-liter reactor.

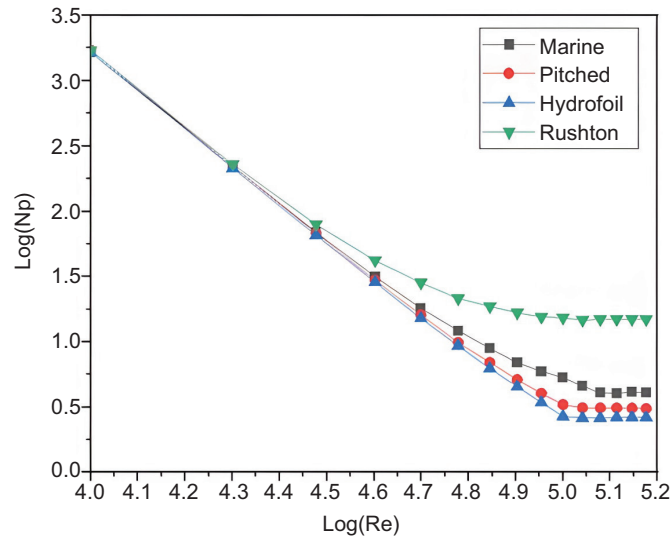


Figure 4. Log (Np) versus Log (Re) ratio for each type of impeller

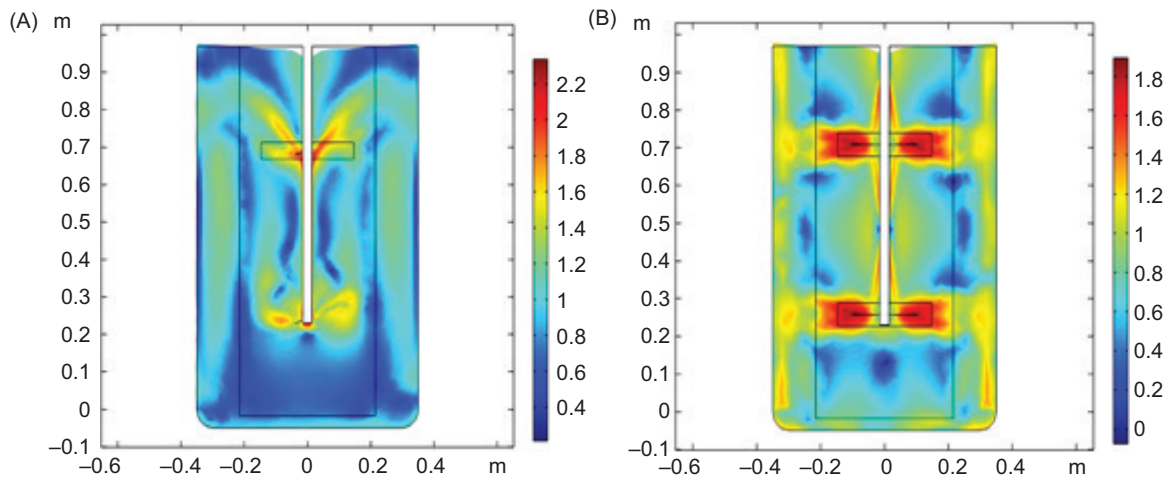


Figure 5. (A) Share rate for Hydrofoil. (B) Share rate for Rushton.

the extraction of proteins from *Spirulina* to the hydrodynamics of the mixture, which must be kept nearly constant to achieve maximum yields. In addition to determining the dimensions for the design of a pilot reactor for the *Spirulina* industry, another contribution of this research is the scaling-up method for a batch reactor, which can serve as a precedent for future research at the industrial level.

At the pilot scale, we could investigate the performance of other agitators that exert high shear forces with the aim of reducing the reaction time. Additionally, this research could continue with the scaling up of the next process: the separation of the solid and liquid phases of the protein solution obtained after stirring where the liquid phase is rich in proteins.

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Author Contributions

All authors contributed equally to this article.

Conflict of Interest

The authors declare no conflict of interest.

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