CHEMICAL ENGINEERING TRANSACTIONS
VOL. 74, 2019
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Sauro Pierucci, Jiří Jaromír Klemeš, Laura Piazza

ISBN 978-88-95608-71-6; **ISSN** 2283-9216
DOI: 10.3303/CET1974013

The Study of the Lipid Extraction Process for the Production of Third-Generation Biofuel from the Pre-Treated Microalgae Chlorella Vulgaris Biomass

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Chlorella is a promising type of microalgae, which can be considered as a source of raw materials for obtaining valuable substances (lipids, carotenoids, chlorophyll, etc.). One of the most energy-intensive stages of production of technical lipids is the extraction stage. The low efficiency of this stage is due to the strong cell wall of microalgae, which prevents the penetration of the solvent into the cell and dissolved lipids from the cell into the volume of the mixture of solvents. In order to increase the efficiency of extraction, cell walls are destroyed, resulting in the formation of three types of cells: "destroyed", "dead but still in shape", "living cells". In the course of theoretical and experimental research the peculiarities and mechanisms of lipid extraction in the system "polar and non-polar solvent" were studied. A mathematical model of the process of lipid extraction from Chlorella microalgae is proposed.

1. Introduction

Microalgae still attract the interest of researchers as a promising object for study (Ma et al., 2018). Individual cells can be considered as bioreactors for obtaining valuable substances (lipids, carotenoids, chlorophyll, etc.), and their ratio can vary in some range depending on the conditions of cultivation (intensity of illumination, periods of alternation of dark and light phases, temperature, composition of nutrient medium, its supply program, biomass concentration, etc.). Chlorella is a promising microalga with high growth rates (under optimal conditions) and resistance to contamination, as it can be cultivated even on wastewater (Dvoretsky et al., 2018). Research is actively under way to reduce the cost of microalgae cultivation and processing (Tredici et al., 2015; Han et al., 2017; Politaeva et al., 2018; Sati et al., 2019). The presence of a strong cell wall is a significant disadvantage that hinders the widespread commercial use of the Chlorella microalgae. This fact leads to increased energy consumption in the implementation of preliminary cell destruction by various methods (using enzymes, ultrasound treatment, electromagnetic field, microwave radiation, etc.) and subsequent extraction. Studies of methods to reduce the cost of extraction of valuable components from microalgae are being actively conducted around the world (Concas et al., 2017; Roux et al., 2017). It should be noted that only a small number of studies aim to develop mathematical models describing the quantitative characteristics of the process of extracting components from microalgae. Mathematical models that take into account the interrelation of processes within the simulated object are powerful tools that are difficult to develop, given the labour-intensive identification of kinetic coefficients, as well as heat and mass transfer coefficients in the range of working areas, but they can boost efficiency when applied. They are indispensable in the process of technology scaling and production design, because they allow to calculate the numerical value of the economic criterion and to choose from a variety of alternative technological solutions the optimal complex "process-apparatus-control system", as well as to take into account the influence of uncertain factors in determining the optimal characteristics of process equipment and its operating regimes at the design stage (Dvoretsky and Dvoretsky, 2014).This work is devoted to the study of the mechanism and development of a

Paper Received: 25 March 2018; Revised: 28 July 2018; Accepted: 29 November 2018

Please cite this article as: Dvoretsky D., Dvoretsky S., Temnov M., Tugolukov E., Akulinin E., Golubyatnikov O., Ustinskaya Y., Eskova M., 2019, The Study of the Lipid Extraction Process for the Production of Third-generation Biofuel from the Pre-treated Microalgae Chlorella Vulgaris Biomass, Chemical Engineering Transactions, 74, 73-78 DOI:10.3303/CET1974013

mathematical model of the process of lipid extraction from Chlorella vulgaris microalgae by a mixture of polar and non-polar solvents.

2. Methods and materials

Chlorella vulgaris IFR C-111 strain was cultivated in the photobioreactor, with the use of Tamiya OPTIMUM medium (Dvoretsky et al., 2015). Cell walls of Chlorella vulgaris microalgae were broken using microwave radiation (power 280 W, radiation frequency 2450 MHz, treatment time 10-30 sec.). The determination of the number of intact cells before and after exposure was carried out by direct counting in the Goryaev chamber; the number of cells which lost viability, but retained their shape was counted by adding methylene blue dye to the biomass and directly counting stained cells in the Goryaev chamber. The number of disrupted cells was the difference in the number of cells before and after exposure (Dvoretsky et al., 2016). Extraction of lipids from microalgae cells was carried out similarly to the Bligh-Dyer method, with slight modifications: ethanol and petroleum ether in the ratio 1:2 (vol.). Estimation of lipids extracted from biomass was done by Zoellner and Kirsch method of determination of total lipids (Zoellner and Kirsch, 1962). Distillation of the solvent was carried out using a rotary evaporator IR-1 M3 at a temperature of distillation 85 °C and the speed of rotation of the flask 65 min^{-1}.

3. The mechanism of lipid extraction

As a result of exposure to microwave radiation at the disintegration stage, some microalgae cells remain intact (group A), some cells retain their shape but lose viability (dead, group B), and some cells are destroyed (group C). Characteristics of microalgae cells of groups A, B and C are presented in Table 1.

Table 1: Characteristics of microalgae cells of different groups

	A	*B*	*C*	Source
Cell size, μm	3–12	3–12	3–12	Bogdanov (2007)
Thickness of cell wall, nm	45	45	45	Gerken et al. (2012)
Aquaporin surface area, m^2	$6.15 \cdot 10^{-20}$	–	–	Holm (2016)
Size of surface area of all cell pores and holes, m^2	$2.6 \cdot 10^{-12}$	$70 \cdot 10^{-12}$	–	Suslov (2014)
Lipid droplet diameter, nm	2–250	2–250	2–250	Koolman and Roehm (2003)
Diameter of protein-lipid complexes, nm	5–80	5–80	5–80	Koolman and Roehm (2003)

In the process of lipid extraction from microalgae cells, three phases were identified: phase 1 - cells (group A intact and group B dead, but retaining shape); phase 2 - mixture of water and polar solvent (ethanol); phase 3 - non-polar solvent (droplets of petroleum ether).

The mechanism of the process of lipid extraction from microalgae cells of different types (Figure 1) can be described as follows.

Intact cells (group A, phase 1). Ethanol causes denaturation of integral and peripheral proteins of the cytoplasmic membrane, which leads to the loss of its efficiency (disruption of cellular activity). Polar solvent molecules diffuse through open aquaporins into cells. When the equilibrium concentration (at the moment of time τ_2) is reached, lipids inside the cell begin to diffuse outwards.

Dead but having retained the shape cells (group B, phase 1). The mixture of water and polar solvent penetrates into the cells through the holes formed in the cell wall as a result of microwave radiation. When the equilibrium concentration (at the moment of time τ_1) is reached, lipids inside the cell begin to diffuse outwards.

Mixture of water and ethanol (phase 2). At the moment of time $\tau = 0$, in phase 2, the lipids in the group C cells will dissolve in droplets of petroleum ether. At the moment of time τ_1 lipids from the cells of group B begin to flow into phase 2, and at the moment of time τ_2 - from the cells of group A.

Petroleum ether droplets (phase 3). The lipids that appeared in phase 2 pass to droplets of petroleum ether. Dispersion interactions (Van der Waltz forces) appear between lipid and petroleum molecules, and lipid molecules begin to diffuse into the droplets of petroleum ether. Since the oil droplet has a high surface tension value ($35.4 \cdot 10^{-3}$ N/m) in comparison with petroleum ether ($18.42 \cdot 10^{-3}$ N/m), a petroleum ether will be located in the border area with the polar solvent (minimum energy principle).

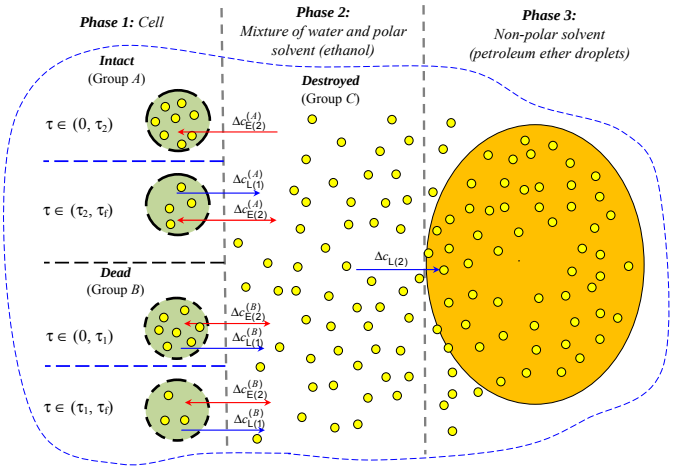


Figure 1: Diagram of the process of lipid extraction from microalgae in the system "ethanol – petroleum ether"

Thus, in the process of lipid extraction from microalgae cells, three stages can be distinguished, characterized by the following balance equations:

1) at $\tau \in [0;\tau_1]$ – $c^{(k)}_{E(1)} = c^{(k)}_{E(1)}(0) + \Delta c^{(k)}_{E(2)}$, $c_{L(1)} = c^{(A)}_{L(1)} + c^{(B)}_{L(1)}$ (here $c^{(k)}_{i(j)}$ is the concentration of *i*-th substance in *j*-th phase for *k*-th form of cells, mol/m^3; E - ethanol, L - lipids; Δ - change of concentration); $c_{E(2)} = c_{E(2)}(0) - \Delta c^{(B)}_{E(1)}$, $c_{L(2)} = c_{L(2)}(0) - \Delta c_{L(3)}$; $c_{L(3)} = c_{L(3)}(0) + \Delta c_{L(2)}$;

2) at $\tau \in [\tau_1;\tau_2]$ – $c^{(k)}_{E(1)} = \Delta c^{(k)}_{E(2)}$, $c_{L(1)} = c^{(A)}_{L(1)} + c^{(B)}_{L(1)} - \Delta c^{(B)}_{L(1)}$; $c_{E(2)} = c_{E(2)}(\tau_1) - \Delta c^{(B)}_{E(2)} - \Delta c^{(A)}_{E(2)}$; $c_{L(2)} = c_{L(2)}(\tau_1) + \Delta c^{(B)}_{L(1)} - \Delta c_{L(3)}$; $c_{L(3)} = c_{L(3)}(\tau_1) + \Delta c_{L(2)}$;

3) at $\tau \in [\tau_2;\tau_f]$ – $c_{L(2)} = c_{L(2)}(\tau_1) + \Delta c^{(B)}_{L(1)} + \Delta c^{(A)}_{L(1)} - \Delta c_{L(3)}$, $c_{L(3)} = c_{L(3)}(\tau_2) + \Delta c_{L(2)}$.

In mathematical description of the process of lipid extraction from microalgae cells, the following assumptions were made: 1) the transfer of the distributed substance through the phase surface is considered as a process of simple diffusion of the substance through the lipid bilayer membrane (Antonov et al., 2003), described by the first Fick's law, in which the concentration gradient is determined by the difference in concentrations in solutions near the membrane surfaces; 2) the humidity of microalgae paste is 98 %; 3) all biomass cells have the same wall thickness of 45 nm (Gerken et al., 2012), radius of 3 μm, surface area and number of intracellular lipids; 4) when cells are destroyed, all intracellular lipids are in intracellular fluid, and 100 % of the lipids can be extracted from the destroyed cells; 5) the size of the droplets of the non-polar solvent (petroleum ether) is 300 μm; 6) each intact cell has the same amount of aquaporins, which have the same size 0.28 nm and the throughput capacity of 10^9 water molecules per 1 s (Karp, 2009); 7) as a result of disintegration, holes appear in the dead cells whose cross-section area is ≈25 % of the total area of the cell wall surface; 8) all biomass cells contain approximately the same amount of intracellular lipids ≈31 % of the cell dry matter; 9) lipid transport through phase 2 is instantaneous; 10) lipids contained in microalgae biomass consist mainly of triolein, tristearin, trypalmithin, so the molecular weight of the average lipid molecule was assumed to be 861 g / mol.

4. The mathematical model

The amount of distributed substance through the phase surface over time $d\tau$ is determined by Eq(1):

$$\frac{dM_{i(j)}^{(k)}}{d\tau} = -D_{\text{int},i} \cdot F_{i(j)}^{(k)} \cdot B_{i(j)}^{(k)} \frac{\left(c_{i(j)} - c_{i(j)}^{(k)*}\right)}{l_j}, \tag{1}$$

where *i* is the designation of the distributed substance: E - ethanol, L - lipids; *j* - phase number, *j*=2.3; *k* - cell shape: *A* - intact, *B* - dead; $c_{i(j)}$ and $c_{i(j)}^{(k)*}$ are concentration and equilibrium concentration of *i*-th substance in *j*-th phase for *k*-th shape of the cell, mol / m^3; D_{int} - molecular diffusion coefficient, m^2 / s; *F* - area of contact of phases, m^2; *B* - correction coefficient, taking into account the area of pores and holes of the cell wall; *l* - membrane thickness, m;

$$\begin{gathered} D_{\text{int},i} = \frac{RT}{N_a}\frac{1}{6\pi\mu r_i};\ F_{i(2)}^{(B)} = y^{(B)} V \pi r_{\text{c}}^2;\ F_{i(2)}^{(A)} = y^{(A)} V \frac{D_{\text{ef}} N_a}{100}\pi r_{\text{p}}^2;\ F_{L(3)} = \frac{3V_{\text{ns}}}{r_{\text{ns}}};\ B_{i(2)}^{(B)} = \frac{1}{4}; \\ B_{i(2)}^{(A)} = \frac{D_{\text{ef}} N_a r_{\text{p}}^2}{4};\ B_{L(3)} = 1; \end{gathered} \tag{2}$$

R - universal gas constant, J / (mol·K); *T* - extraction temperature, *T*=323 K; N_a - Avogadro's number, mol^{-1}; μ - dynamic viscosity, Pa·s; r_i - molecule radius of *i*-th substance, m; r_{c} , r_{p} , r_{ns} - radii of a cell, cell pores and non-polar solvent, respectively, m; *y* - concentration of cells in microalgae paste, mln cells / m^3; V , V_{ns} - volume of microalgae paste and non-polar solvent, respectively, V =330·10^{-6} m^3, V_{ns} =186·10^{-6} m^3; D_{ef} - coefficient of water diffusion inside the cell, D_{ef} = 1.2·10^{-9} m^2/s.

In accordance with the accepted assumptions, the mathematical description of the kinetics of lipid extraction from microalgae cells takes the following generalized form (3):

$$\begin{cases} \dfrac{dc_{\text{E}(2)}}{d\tau} = -\dfrac{1}{V}\left(\dfrac{dM_{\text{E}(2)}^{(A)}}{d\tau} + \dfrac{dM_{\text{E}(2)}^{(B)}}{d\tau}\right), \\ \dfrac{dc_{\text{L}(2)}}{d\tau} = \dfrac{1}{V}\left(\dfrac{dM_{\text{L}(2)}^{(A)}}{d\tau} + \dfrac{dM_{\text{L}(2)}^{(B)}}{d\tau} - \dfrac{dM_{\text{L}(3)}}{d\tau}\right), \\ \dfrac{dc_{\text{L}(3)}}{d\tau} = \dfrac{1}{V}\left(\dfrac{dM_{\text{L}(3)}}{d\tau}\right), \tau \in [0;\tau_f] \end{cases} \tag{3}$$

with the initial conditions:

$$c_{\text{E}(2)}(0) = c_{\text{E}(2)}^{\text{in}},\ c_{\text{L}(2)}(0) = c_{\text{L}(2)}^{\text{in}},\ c_{\text{L}(3)}(0) = 0,\ \tau \in [0;\tau_1], \tag{4}$$

$$c_{\text{E}(2)}(\tau_n) = c_{\text{E}(2)}^{\text{out}}(\tau_n),\ c_{\text{L}(2)}(\tau_n) = c_{\text{L}(2)}^{\text{out}}(\tau_n),\ c_{\text{L}(3)}(\tau_n) = c_{\text{L}(3)}^{\text{out}}(\tau_n),\ n = 1,2,,\ \tau \in [\tau_1;\tau_2],\ \tau \in [\tau_2;\tau_{\text{f}}], \tag{5}$$

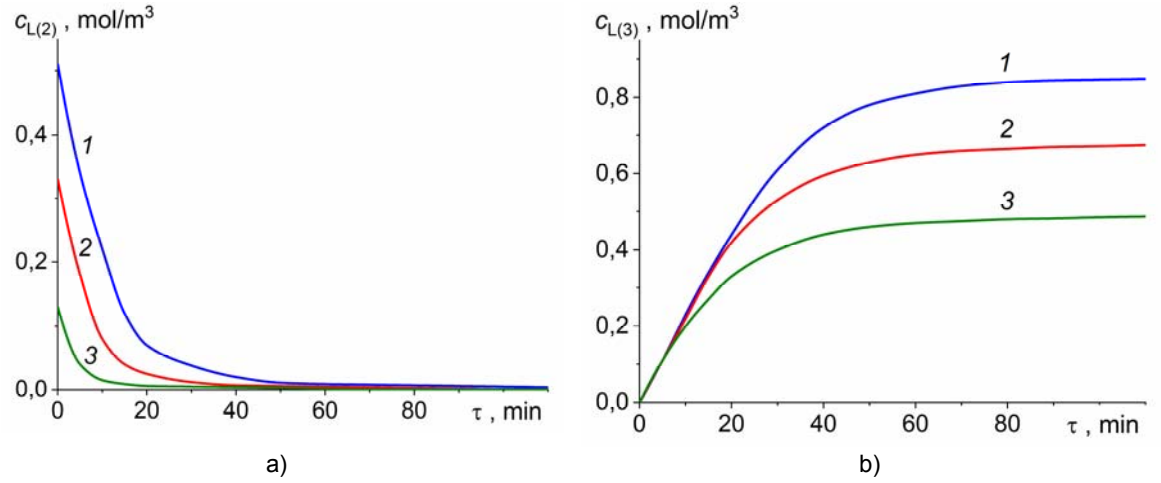
Where τ is the duration of the extraction process, s. For the extraction time interval $\tau \in [0;\tau_1]$ in the system of equations (3) $\dfrac{dM_{\text{L}(2)}^{(A)}}{d\tau} = 0$, $\dfrac{dM_{\text{L}(2)}^{(B)}}{d\tau} = 0$; at $\tau \in [\tau_1;\tau_2]$, $\dfrac{dM_{\text{E}(2)}^{(B)}}{d\tau} = 0$, $\dfrac{dM_{\text{L}(2)}^{(A)}}{d\tau} = 0$; at $\tau \in [\tau_2;\tau_{\text{f}}]$, $\dfrac{dM_{\text{E}(2)}^{(A)}}{d\tau} = 0$, $\dfrac{dM_{\text{E}(2)}^{(B)}}{d\tau} = 0$.

The values of the model parameters (1)-(5) are given in Temnov (2017). The values of equilibrium concentrations obtained as a result of experiments were as follows (mol / m^3): $c_{\text{E}(2)}^{(A)*}$ = 1.6·10^7, $c_{\text{E}(2)}^{(B)*}$ = 1.6·10^7, $c_{\text{L}(2)}^{(A)*}$ =0.12, $c_{\text{L}(2)}^{(B)*}$ =0.24, $c_{\text{L}(3)}^{*}$ =0.87.

The calculated and experimental data were obtained at values of τ_1=20 min, τ_2=50 min, τ_{f} =110 min. The accuracy analysis of the mathematical model (1)-(5) was performed using Fisher's F-criterion for the significance level of 0.05 (Rees, 2001). To solve the system of ordinary differential equations (1)-(5) Runge-Kutta method of the 4th order of accuracy was used (Rice and Do, 2012) in Matlab.

5. Results and discussion

Figure 2 shows lipid concentration curves calculated with the model (1)-(5) for phase 2 and phase 3 at different ratios of intact ("A"), dead ("B") and destroyed ("C") cells in the biomass, (%): "curve 1" - 40:32:28, "curve 2" - 60:22:18, "curve 3" - 80:13:7. The samples were obtained by microwave destruction of cells for 30 s, 20 s and 10 s, respectively. The analysis of the graphs shows that the reduction of the proportion of intact cells in the biomass by 2 times (from 80 to 40%) allows increasing the initial concentration of lipids in the phase 2 by ~4 times (from 0.13 to 0.51 mol / m^3, Figure 2a, curves 1, 3), and the final concentration of lipids in the phase 3 by ~1.8 times (from 0.48 to 0.85 mol / m^3, Figure 2b, curves 1, 3).



a) b)

Figure 2: Changes in lipid concentration (a)- in phase 2 and (b) – in phase 3.

At the first stage of extraction, the rate of lipid uptake from intracellular fluid into the petroleum ether is the same and equals ~0.02 mol / (m^3·min) (Figure 2a, curve 1, interval 0-20 min). This is explained by the fact that this process is limited by molecular diffusion of lipids in the droplets of petroleum ether. At the same time, the exhaustion of lipids in phase 2 for cases 2, 3 (Figure 2a, curves 2, 3) occurs before the 20 min point because of the lower initial concentration of lipids compared to case 1 (Figure 2a, curves 1-3, interval 0-20 min). At the second stage of extraction (from 20 to 50 min), lipids from dead cells are diffused into phase 2, which are immediately (according to assumption 9), passing through phase 2 and further, diffused into the petroleum ether. As a consequence, there is an increase in the lipid concentration in phase 3 (Figure 2b). At the third stage of extraction (from 50 to 110 min), there is also an increase in the concentration of lipids in phase 3 due to the diffusion of lipids from intact and dead cells into phase 2. Different rate of lipid accumulation in phase 3 for three cases is explained by the different ratio of intact and dead cells in the biomass.
The results are consistent with McConnell and Farag (2013), where the kinetics of lipid extraction from Chlorella vulgaris biomass was investigated. It was determined that depending on the conditions of extraction, 70-80 % of lipids were extracted within 20-50 minutes.

6. Conclusions

As a result of the study of the lipid extraction process in the polar and non-polar "ethanol-petroleum ether" solvent system, the mechanism for extracting lipids from the biomass of the Chlorella microalgae pre-treated by microwave radiation was considered. A mathematical model of lipid extraction was proposed, taking into account the average size of microalgae cells and calculating the kinetics of the process. A satisfactory concordance of experimental and calculated data using Fisher's F-criterion for the significance level of 0.05 was achieved. The discussed mechanism and the developed model can be used for technology scaling and for building mathematical models for optimal design of industrial extractors, which additionally requires consideration of hydrodynamics of flows in the extractor.

Acknowledgments

The work was commissioned and carried out with financial support of the Ministry of Education and Science of the Russian Federation.

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