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Research Article

Medium Optimization by Artificial Neural Networks for Maximizing the Triglycerides-Rich Lipids from Biomass of *Chlorella vulgaris*

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ABSTRACT

Artificial neural networks (ANN) have been widely accepted for different microalgal biotechnology aspects due to their versatile applications and advantages. A naturally isolated strain of *Chlorella vulgaris*, was investigated to assess the influence of three major culture medium constituents including glucose (6-18^{-1} g L), nitrate (0.5-4.5 g L^{-1}) and phosphate (0.01-0.08 g L^{-1}) sources on lipid production using artificial neural network (ANN) method. A feed forward method was used. The most significant parameter for lipid production was found to be carbon concentration. The highest amounts of lipid production (1.944 g L^{-1}) and biomass productivity (0.31 g L^{-1} d^{-1}) was observed in the optimized medium condition composed of glucose (15 g L^{-1}), nitrate (1.04 g L^{-1}) and phosphate (0.005 g L^{-1}). The suitability and robustness of using ANN methods for optimization process in microalgal biodiesel production assessment was indicated. Moreover, palmitic acid (C16:0) and linoleic acid (C18:2) were the most abundant fatty acids in the studied *C. vulgaris* strain under mixotrophic mode of cultivation.

Keywords: Artificial neural networks, Biomass, *Chlorella vulgaris*, Medium optimization, Triacylglycerol-rich lipids.

INTRODUCTION

The increasing worldwide demands for energy, global warming phenomenon and dwindling fossil fuel resources has motivated the worldwide attempts to find alternative energy supplies[1]. Sugars and vegetable oils from arable crops as the first-generation of biofuels and the lingo-cellulosic biomass feedstocks as the second-generation of biofuels have faced several shortcomings. The oleaginous microalgae as the third-generation of biodiesel producers could be served as fascinating alternative resource for bioenergy production[2]. The produced biodiesel from microalgal biotechnology offers several advantages over their competing alternatives. It is a clean and renewable source with better biodegradability and more suitable flash points, containing lower amounts of sulfur with no increasing the total release of carbon dioxide[3]. Besides, due to current achievements in genetic and bioprocess engineering approaches which maximizes the biodiesel yields and improves the biodiesel properties and quality, its exploitation as a sustainable bioresource has become more feasible and cost-effective. Furthermore, the current achievements in metabolic engineering of microalgae as the newly emerged fourth-generation of biofuels[4], and their genome sequencing has opened new avenues towards microalgal systems and synthetic biology. Among various studied microalgal sources for green energy production, *Chlorella vulgaris* has presented a great promise for large scale biodiesel production. It is a unicellular spherical-shaped green microalga form Chlorophyta division. Owing to its higher growth rates, simple and cheap culture, and higher lipid contents, *Chlorella* could be considered as a potential biodiesel producer[5]. The cost-benefit biodiesel production from microalgae has a close relationship with the growth rates of the strain, its biomass productivity and also lipid metabolism and its fatty acid profile. Moreover, the biomass production and lipid accumulation is defined by various parameters such as the nutrients concentration in the culture medium, temperature, and light. The type of carbon, nitrogen and phosphorus supply and also their amount in the culture medium causes main influences on biomass production. Because these macronutrients, play a crucial role in different biosynthetic pathways in microalgae and the required energy for the cells to perform their physiologic tasks and biomass production is from that. In the present study, a multi layered feed-forward model was designed based upon the ANN and was used to optimize the culture medium conditions to maximize the lipid contents in *C. vulgaris* biomass. The input parameters, were the glucose, nitrate and phosphate concentrations in the culture medium. Moreover, the obtained fatty acid profile of the studied microalgal strain after optimization study was also determined.

MATERIALS AND METHODS

Microalgal source and culture medium

Unicellular microalga *C. vulgaris* was attained from the Microalgal Culture Collection of Shiraz University of Medical Sciences (MCCS), Shiraz, Iran. BG11 culture

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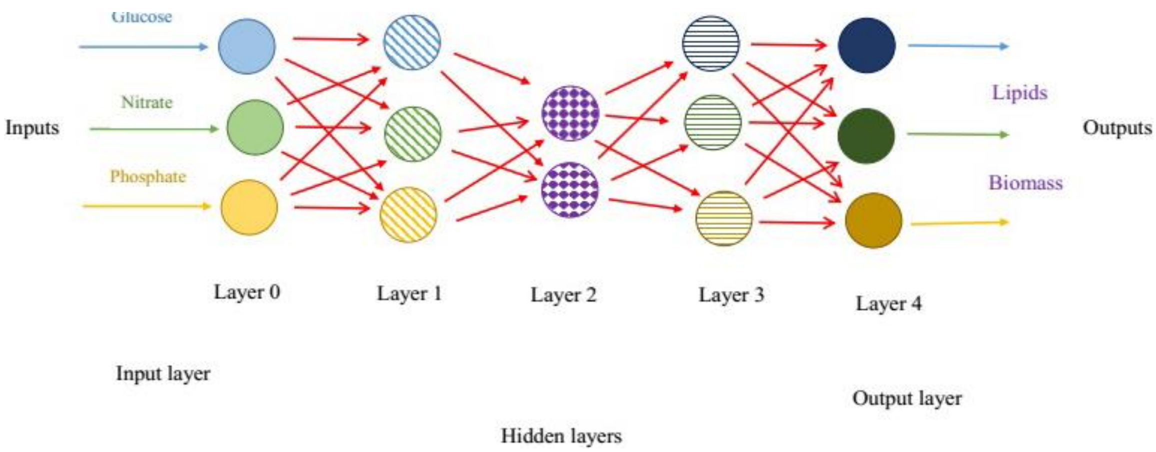

Figure 1: Schematic view of the exploited feed forward multi layered artificial neural networks paradigm for this optimization study.

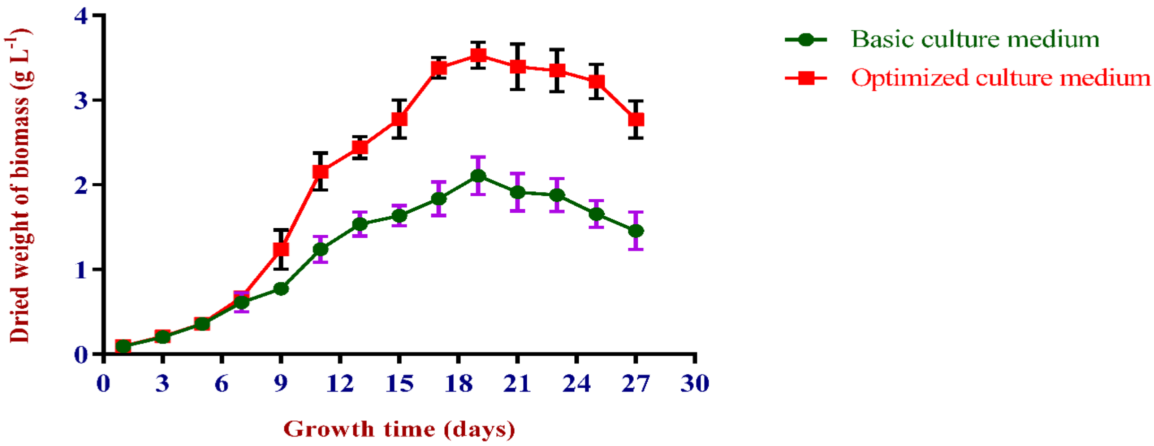

Figure 2: Growth pattern of *C. vulgaris* under mixotrophic cultivation mode in basic and optimized BG11culture medium during 28 days of study using dry cell weight method (g L^{-1}).

medium (pH = 8.0)[6], was employed for preservation and cultivation.

Culturing conditions for microalgal growth

The cultivation procedure was performed for 28 days of study. *C. vulgaris* cells were cultivated in 500 mL Erlenmeyer flasks at 25 °C in an orbital incubator shaker (PECO, Iran) with the following cultivation conditions: agitation rate at 130 rpm, light intensity of 60 mol m^{-2} s^{-1}, working volume of 100 mL and the initial pH of 8.0. To monitor the microalgal growth, random samples were obtained from the culture media aseptically every two days using dry cell weight method[7].

Design experiment using ANN

To harness the artificial neural networks for optimization of triacylglycerol-rich lipid production process, MATLAB software and neural network toolbox, release 2013a (MathWorks, Inc., Natick, Massachusetts, US) was employed to develop a multi layered feed forward paradigm (Fig. 1) was developed. Three distinct input neurons were represented the three different studied parameters including glucose, nitrate and phosphate levels in the culture medium (Table 1). Besides, one layer of hidden artificial neurons and also an output neural network representing the amount of lipid production were included in the study. The total amounts of required neurons to construct the hidden layers was determined using try and error method. The minimum amount of required artificial neurons to construct the final model was defined to be 10 neurons. After it, 70% of the obtained data from experimental part were used for training the networks and the remaining parts of the experimental data (30%) were employed to examine and validate the designed model.

Analytical methods

The total lipid content of the studied microalga in every experiment was extracted using methanol/dichloromethane (2:1, v/v) mixture containing butylated hydroxytoluene (0.5 mg) and quantified gravimetrically[8]. Each lipid extraction experiment repeated for three times and the mean values were reported. The significance of the results (*n*=3) was measured using ANOVA (IBM SPSS, version 22.0, Armonk, NY: IBM Corp.). The observed difference at 5% were regarded as significant. To analyze the fatty acid composition form *C. vulgaris* biomass a previously adopted protocol was used[9].

RESULTS AND DISCUSSION

The exploited feed forward artificial neural networks analysis provided a robust model for prediction the best operational conditions to achieve the highest amounts of triacylglycerol-rich lipids in *C. vulgaris* culture. The results suggested that a BG-11 culture medium enriched with 15.0 g L^{-1} of glucose, 1.04 g L^{-1} of nitrate and 0.005 g L^{-1} of phosphate will be resulted to 1.97 g L^{-1} and 3.59 g L^{-1} of lipids and biomass respectively, as their highest values. On the other words, the amounts of lipid and biomass in the optimized cultivation condition could be improved up to 2.59 and 1.71 folds than their initial values in the basic cultivation conditions. After it, an extra experimental study was performed using the suggested concentrations for glucose, nitrate and phosphate to assess the validity of the provided model. After implementing the extra experiment using the suggested values for culture medium constituents, the maximum amounts of 1.94 g L^{-1} and 3.53 g L^{-1} were reached for lipid and biomass accordingly. The observed trends showed 98.48% and 98.33% similarity with the predicted values, namely. Hence, the validity and robustness of the employed ANN method were confirmed. To monitor the growth trend of *C. vulgaris* strain, to different methods including direct counting and cell dry weight methods were used (Fig. 2). Triacylglycerol-rich lipids were also analyzed using vanillin sulfuric acid method and the obtained data were expressed as gram liter of the culture medium. The growth trend of *C. vulgaris* cells in both experiments exhibited a sigmoidal pattern with a lag phase period during first 4 days following by a significant logarithmic (exponential) phase from the 4^{th} to 16^{th} day of experiment and also a noticeable stationary phase of growth after 16^{th} day of experiment. Moreover, the growth kinetics, biomass and lipid production and productivities were also compared in basic non-optimized condition with the obtained data from optimized conditions. The maximum biomass concentration (X_{max}) and specific growth rate (μ_{max}) of *C. vulgaris* in optimized BG-11 culture medium (3.530 g L^{-1} and 0.31 d^{-1}) was shown to be higher than their initial values (2.107 g L^{-1} and 0.25 d^{-1}) in the basic culture medium, namely. On the other hand, the maximum cell density (N_{max}) (which was achieved (D_{max}) in the 19^{th} day of cultivation) in the optimized cultivation condition (0.614 x 10^6 cell mL^{-1}) seem to be higher than the observed cell number in the basic culture medium (0.432 x 10^6 cell mL^{-1}). The maximum biomass productivities (P_{max}) in basic and optimized culture media were also determined to be 0.27 and 0.41 g L^{-1} d^{-1}, namely. After 28 days of microalgal cultivation, the ultimate concentration of lipids (L_{max}) were achieved to 0.761 and 1.944 g L^{-1} in basic and optimized conditions respectively (Table 2). The X_{max}; μ_{max}; P_{max} and L_{max} values in optimized culture medium was 1.67; 1.24; 1.52 and 2.55 fold higher than the initial culture medium respectively. In recent years, application of different artificial neural network-based methods has attained vast acceptability to develop the non-linear experimental models and specially in the cases in which the application of an experimental model is impossible[10]. Among different artificial intelligence-based methods, the artificial neural networks have gained more attention[11]. Generally, for optimization of a biological process, the exploitation of ANN methods offers more advantages such as ability to build more complex models, more accuracy and flexibility in modeling the non-linear conditions compared to response surface methodology (RSM) methods[12]. In some previous studies, the ANN method has been employed simultaneously with another design of experiment methods such as RSM for optimization of different biological processes to evaluate the advantages of ANN methods in practice[13]. To examine the applicability of using microalgal lipids for biodiesel production, the fatty acid composition of the obtained lipids is of importance[14]. Mainly, fatty acid methyl esters composed of 16 to 18 atoms of carbon are considered as a suitable choice for green energy production. Various types of fatty acids such as saturated fatty acids, mono unsaturated fatty acids and also poly unsaturated fatty acids were detected in *C. vulgaris* lipids. Myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0) were noticed as the main saturated fatty acids. Besides, some fascinating monounsaturated fatty acids such as palmitoleic acid (16:1), a monosaturated ω-7 fatty acid and oleic acid (18:1), a monosaturated ω-9 fatty acid were also detected in the analyzed microalgal lipids. Furthermore, some polyunsaturated fatty acids comprising of di and tri unsaturated fatty acids were found to be as hexadecadienoic acid (16:2), linoleic acid (18:2) a poly unsaturated ω-6 fatty acid, α-linolenic acid (18:3n3), an essential ω-3 fatty acid and γ-linolenic acid (18:3n6), an important ω-6 fatty acid were identified as the major detected polyunsaturated fatty acids in the studied *C. vulgaris* strain. It could be comprehended that several important fatty acids such as oleic acid, stearic acid and γ-linolenic acid with pharmaceutical and nutritional importance are occurring in the studied *C. vulgaris* strain. Nowadays, the optimization methods have been largely exploited in biotechnology and pharmaceutical sciences. The current optimization methods are regarded as response surface methodology and simplex optimization. On the other hand, with increasing application of computer-based models in different areas of process optimization, some new challenges have been risen which cannot be hampered using the conventional methods. In this content, the application of newly emerged optimization methods such as simulated annealing[15], genetic algorithms[16], artificial intelligence-based methods (AI) and more importantly, artificial neural networks (ANN) could be of help[17].

Inspired by the human nervous system performance, ANN is considered as an information processing platform which could be of help for solving the complex problems.

Table 1: Independent variables; their coded and actual values used for optimization study.

Independent variable	Units	Symbol	Code levels		
			-1	0	1
Glucose	g L^{-1}	X_1	6	12	18
KNO_3	g L^{-1}	X_2	0.5	2.50	4.5
KH_2PO_4	g L^{-1}	X_3	0.01	0.04	0.08

Table 2: The growth parameters, maximum lipid and biomass production and productivities observed in *C. vulgaris* under mixotrophic cultivation mode in basic and optimized culture conditions during 28 days of study.

Culture medium	Growth parameters*		Cell number		Biomass productivity	Lipid concentration
	X_{max}	μ_{max}	N_{max} (x 10^6 cell mL^{-1})	D_{max}	P_{max}	L_{max}
Basic	2.107	0.25	0.432	19	0.27	0.761
Optimized	3.530	0.31	0.614	19	0.41	1.944

*X_{max}= maximum biomass concentration (g L^{-1}); μ_{max} = maximum specific growth rate (d^{-1}); N_{max}= maximum cell number; D_{max}= the day with maximum N_{max}; P_{max} = maximum biomass productivity (g L^{-1} d^{-1}); L_{max}= maximum concentration of lipids (g L^{-1}).

CONCLUSION

Findings of this study indicated that that ANN might be effectively used to optimize the culture medium conditions in mixotrophic culture of *C. vulgaris* for biomass and especially triacylglycerol-rich lipids production. Furthermore, it was indicated that three observed parameters including glucose, nitrate and phosphate concentration in the culture medium, exhibit a great contribution on lipid production in the mentioned microalgal strain. The optimal cultivation condition for *C. vulgaris* was suggested (g L^{-1}): glucose (15.0), KNO_3 (1.04), and KH_2PO_4 (0.005). the other elements of the culture medium could be remained to their initial concentrations. Besides, it was implied that the mixotrophic culture of *C. vulgaris* could be considered as a promising method for biodiesel and triacylglycerol-rich lipids production.

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