OPTIMIZING GROWTH AND ECONOMIC VIABILITY OF *CHLORELLA VULGARIS* USING VARIOUS NITROGEN AND CARBON SOURCES UNDER LABORATORY CONDITIONS

Mohamed S. M. Abdel Kader(1); Hesham I. Al-Kassas(1); Abo Al-Khair B. Al-Sayed(2) Abdullah M. Abdel Maksoud(3); Ahmed M. Abdel Aziz(4)

1) Department of Environmental Agricultural Sciences Faculty of Post Graduate Studies and Environmental Research, Ain Shams University, Cairo, Egypt 2) National Research Center, Dokki, Giza, Egypt 3) Faculty of Agriculture, Ain Shams University, Cairo, Egypt 4) Department of Environmental Economics, Legal and Administrative Sciences, Faculty of Post Graduate Studies and Environmental Research, Ain Shams University, Cairo, Egypt

ABSTRACT

The green alga *Chlorella vulgaris* was cultivated in a laboratory setting using various nitrogen and carbon sources to assess its growth potential and identify the most economical carbon source. Initially, the alga was grown under optimal conditions using the BG-11 medium, with nitrate (NO_3) and ammonia (NH_3) tested at 17.6 mM N to establish growth curves under ambient conditions. Subsequently, the alga was incubated with urea, organic carbon (vinasse waste), ammonium carbonate, and sodium acetate, based on an initial urea carbon content of 106 ppm. Experiments were conducted in a fully transparent 108-liter Plexi-Glass photobioreactor, with dry weight and pigment content measured as key parameters. The biomass was also analyzed for economic viability. Results indicated that both nitrogen sources had similar effects on dry weight and carotenoid content, however, nitrate significantly increased chlorophyll content. Among carbon sources, organic carbon enhanced dry weight and carotene content, while sodium acetate boosted chlorophyll content. The highest dry weight (1.72 g/L) was achieved with organic carbon, and the maximum chlorophyll content (62.89 mg/L) was obtained with sodium acetate. Organic carbon also yielded 29.19 mg/L of carotenoids. Economic analysis revealed that organic carbon had the lowest production cost per kilogram of *Chlorella vulgaris* biomass under mixotrophic conditions.

Key words: *Chlorella vulgaris*, nitrogen source, carbon source, growth, chemical analysis, economic evaluation

INTRODUCTION

Carbon nutrition is a critical factor in the cost-effective mass production of algae. Algae are known to contain over 50% carbon by dry weight and can fix atmospheric carbon dioxide at rates up to 40 times higher than terrestrial plants. However, the low concentration of atmospheric carbon dioxide (0.036%) poses a significant challenge for commercial-scale algae production **(Chisti, 2007).** To address this, supplemental carbon sources are essential for optimal growth and productivity.

Both organic and inorganic carbon sources are widely used in algae cultivation. Inorganic sources, such as bicarbonate and carbonate salts, are often expensive and inefficient due to chemical decomposition, high salt content, and potential losses as carbon dioxide or carbonate precipitates. Food-grade carbon dioxide, while effective, is costly, and industrial carbon emissions, though cheaper, present safety and environmental hazards. In contrast, organic carbon sources are more stable, cost-effective, and easier to utilize. They often contain essential macro- and micronutrients, as well as growth-promoting compounds such as organic acids, amino acids, and phytohormones. Industrial food wastes, such as citrate wastes(**El-Sayed, 2010 and El-Sayed *et al.*, 2012),** okara **(Sheraze *et al.*, 2017),** bagasse **(El-Sayed *et al.*, 2020; Nashwa *et al.*, 2022 and El-Sayed *et al.*, 2022)**, whey **(Asmae et al., 2023a and 2023b),** cassava(**Ogbo, 2010**), corn steep liquor (**El-Sayed *et al.*,2015),** and date palm(**El-Awady *et al.*, 2020**), **(Holanda *et al.*, 2022)**, have emerged as promising organic carbon sources for algae production.

From an environmental perspective, rapid industrial development has led to increased carbon emissions, contributing to global warming and the greenhouse effect. Algae cultivation offers a dual benefit: mitigating hazardous emissions while producing economically valuable biomasses. Under optimal conditions, carbon dioxide or other carbon sources are incorporated into various cell metabolites, with a primary focus on increasing protein content. Conversely, under unfavorable conditions, there is a significant accumulation of carbohydrates and oils. The ideal carbon source should ensure a high fixation rate, be cost-effective, have minimal associated hazardous minerals, and remain stable in the growth medium to prevent loss as carbon dioxide to the surrounding environment. The aim of study underscores the challenges and considerations in designing an effective carbon delivery system for commercial algae production, which is critical for achieving high productivity and economic feasibility.

MATERIALS AND METHODS

Alga, growth medium and conditions

The green alga Chlorella vulgaris, isolated locally, was cultivated in the laboratory to generate sufficient inoculum. Growth was carried out under fully optimized conditions using the standard BG-11 growth medium **(Stainer *et al.*, 1971).** Light was provided from one side light bank (120µ*E*) and aerated by a gentile stream of air / carbon dioxide mixture (1.5%). The grown cultures were then harvested by laboratory centrifuge, washed three times with bi-distill water and then used for the next inoculation. Assessment of *Chlorella vulgaris* growth and potential productivity was performed in 36 mm Plexi-Glass water bath photobioreactor (Fig. 1) controlled in all growth conditions (light, temperature and turbulence). The results were obtained from 3 replicates, and the daily measuring of growth parameters was done (**El-Sayed and Almutairi, 2024).**

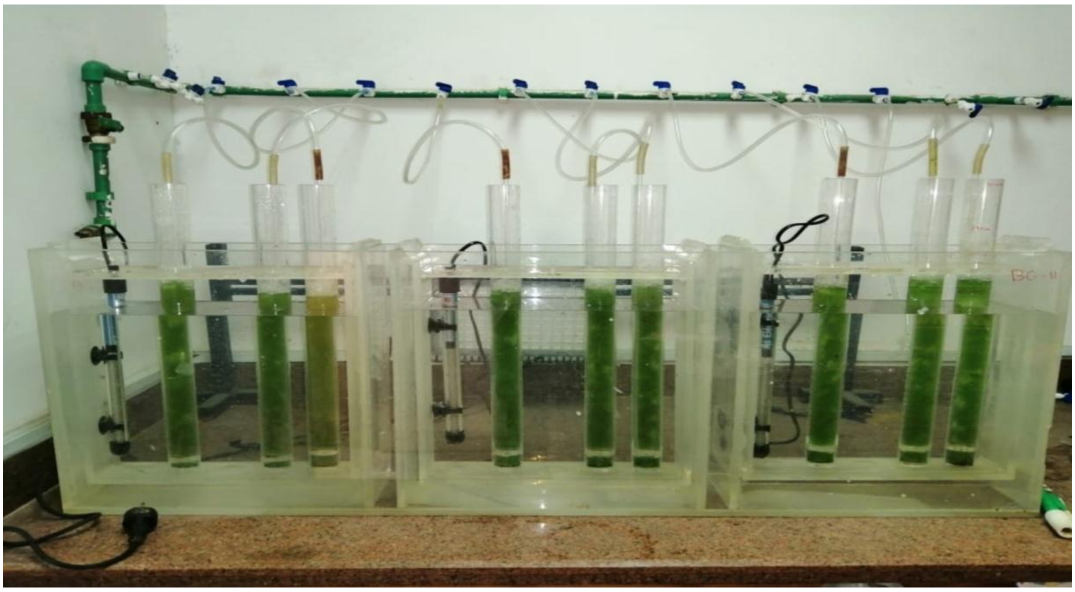

Fig. 1. Water bath controlled photobioreactor

Carbon/nitrogen sources

The first experiment was created under two different sources of nitrogen to assess whether the primary carbon content of urea can fill the algae's need for carbon instead of nitrates and compressed CO_2. The second experiment was done aiming at growing alga under different carbon sources including organic carbon (vinasse); sodium acetate and

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ammonium carbonate verses urea (0.53g.l^{-1}). It should be mentioned here that nitrate, the sole nitrogen source, was substituted by urea at the same nitrogen content (17.6 mM N) to eliminate the accompanied sodium ions effect with partially content of carbon. The used concentrations were calculated based on the commonly used concentration of urea (106 ppm). Carbon sources, initial content (%) and their quantities listed in Table (1).

Table 1. Initial carbon content (%) of different carbon sources used

CARBON SOURCE	UREA	O.C	S.A	A.C
Quantity (g)	0.53	1.06	0.362	0.689
Initial carbon (ppm)	106	106	106	106
Initial carbon (%)	20	10	40	15.19

O.C= Organic carbon, S.A= Sodium acetate and A.C= Ammonium carbonate

Growth measurements

During the whole incubated time, the periodically measured growth parameters were dry weight (g.l^{-1}); total chlorophyll (mg. l^{-1}) and total carotenoids (mg. l^{-1}). Dry weight was determined by precipitating a define volume of algal slurry over pre-weighted membrane filter (0.45um). The weight differences monitored the obtained biomass within the defined growth period. Pigments were extracted by 95% dimethyl sulfoxide (DMSO) and absorbed at 666 nm according to **Boussiba *et al.* (1992)** for chlorophyll determination; while carotenoids were measured at 446 nm after saponification by alcoholic potassium hydroxide, neutralization by acetic acid and extraction by DMSO.

Harvesting, drying and chemical analysis

When cultures reached their maximum growth, aeration was turned off for 24 hours to allow biomass settling and then the upper clear layer was removed and then cultures were dewatered using laboratory centrifuge (4000 rpm). The drying was performed using circulated oven (105^{o}C) and the dried biomass was then milled to fine powder and kept for the next chemical analysis. Chemical analysis including cell metabolites (protein, carbohydrates and oils) as well as ash and ash fraction was performed based on the adopted methods of **Chapman and Pratt (1978).**

Growth analysis

Growth analysis was done based on the methods of **Pirt, (1973)** for the determined growth parameters.

RESULTS

Growth curve of *Chlorella vulgaris* under nitrate and ammonia nitrogen

As shown in Fig. 2, nitrate grown cultures surpass those of grown with urea nitrogen. During the whole incubation period, a linear relationship between growth as dry weight and advanced age had occurred.

However, growth exposed some inhibitory responses at the late time of incubation. Both nitrogen sources were advised to be used in *Chlorella* nutrition. Furthermore, maximum yield of algal biomass was obtained with urea nitrogen by day 21 and recorded as 1.54 g. l^{-1}.

Growth as dry weight analysis (Table 2) confirmed the superior effect of nitrate nitrogen in chlorella dry weight accumulation, where growth rate was recorded with nitrate nitrogen as 0.1012 g. l^{-1}. d^{-1} compared with 0.0973 with ammonias nitrogen. However, doubling time was lower in nitrate one, degree of multiplication was the maximum with urea nitrogen. It was also found that growth rate was maximized by the early growth period revealed that the optimum time of incubation could serve by day 12 of incubation with urea nitrogen and with day 9 of nitrate nitrogen. Such data seems to have a significant impact on the economy due to the power and labor costs

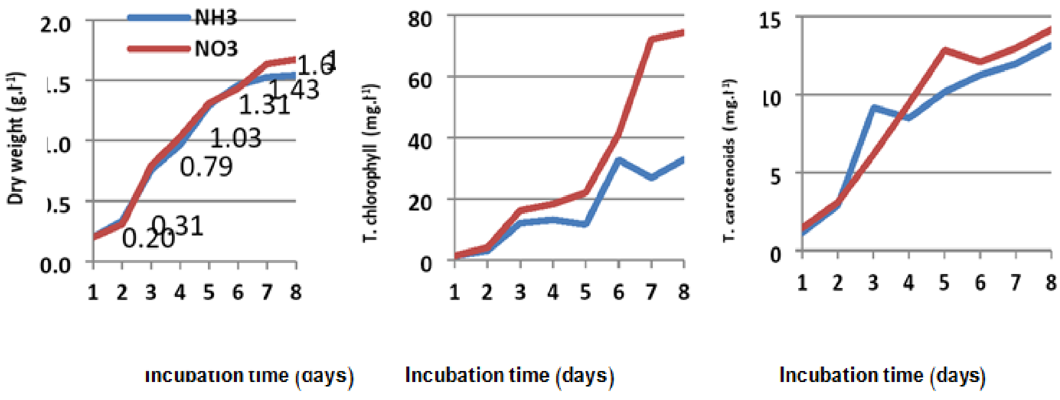

Fig. 2 . Growth metabolites curve of *Chlorella vulgaris* grown under nitrate and ammonias nitrogen.

Three-day' time frequency refers to the interval at which measurements or observations were taken during the experiment. Specifically, it means that data (e.g., dry weight, total chlorophyll, total carotenoids) were recorded every 3 days throughout the incubation period. A 3-day interval strikes a balance, capturing meaningful changes without excessive effort.

Table 2. Dry weigh growth analysis of the water bath grown *Chlorella vulgaris* under nitrate and ammonias nitrogen.

N SOURCE	μ			G			N		
	D.W	**T.Ch**	**T.Car**	**D.W**	**T.Ch**	**T.Car**	**D.W**	**T. Ch**	**T. Car**
Urea	**0.097**	**0.151**	**0.116**	**7.12**	**76.95**	**99.9**	**6.85**	**32.53**	**12.1**
KNO_3	**0.101**	**0.19**	**0.108**	**6.85**	**116.3**	**203.1**	**3.06**	**92.48**	**9.66**

μ= growth rate, G= doubling time, n = degree of multiplication, D.W= Dry weight, T.Ch = Total chlorophyll and T. Car = Total carotenoids

However, doubling time was lower in nitrate one, degree of multiplication was the maximum with urea nitrogen. It was also found that the growth rate was maximized by the early growth period revealed that the optimum time of incubation could serve by day 12 of incubation with urea nitrogen and with day 9 of nitrate nitrogen. Such data seems to be more important in the economy due to the power and labor costs.

When data were subjected to chlorophyll content, marked variation was observed between the two examined nitrogen sources.

Growth analysis as total chlorophyll represented the increase of growth rate due to nitrate utilization as a nitrogen source with low generation time and high multiplication rate. As for carotenoids content under nitrate and ammonias nitrogen, *Chlorella vulgaris* exhibited a slight difference in carotenoids content as grown under the nitrogen sources and a slight increase with nitrate nitrogen might attributed to the initial potassium ion, while carbon resulted in the lowest one.

Growth curve of *Chlorella vulgaris* under different carbon sources:

Among the different examined carbon sources in the current study, organic carbon (vinasse) resulted in the maximum growth dry weight (Fig. 3). It was followed by urea, ammonium carbonate and ended by sodium acetate.

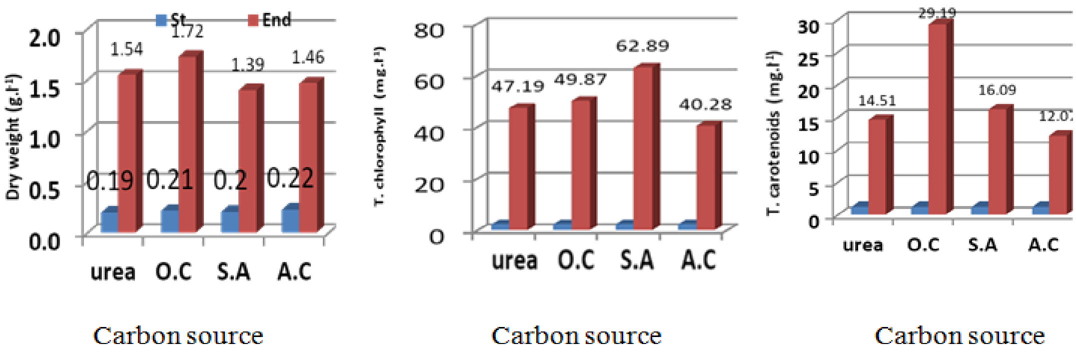

Fig. 3. Growth metabolites curve of *Chlorella vulgaris* grown under different carbon sources.

Inputs required for algae production varied due to location species nutritional mode and growth unit. However, the current trial was achieved on micro-scale (250 ml); the required fixed assets including inoculum and nutrition not differed. On the other hand, light and aeration were mitigated. Table 4 shows the different inputs in concern in and out-doors production.

Table 3. Fixed and variable assets of algae production

FIXED ASSETS	Q	COST (LE)	VARIABLE ASSETS	Q	COST (LE)
Indoor					
Lab	**1**	**6000000**	**Nutrients**	**1**	**30/kg**
Growth unit	**20**	**400000**	**Light**	**1**	**250/day**
Machinery	**2**	**20000**	**Aeration**	**1**	**200/day**
Land	**Variable**	**Variable**			
Yield (kg)	**0.5 kg/day**				
Outdoor					
Land	**Acre**	**Variable**	**Nutrients**	**1**	**15/kg**
Construction	**1**	**2000000**	**Light (natural)**	**1**	**50**
Water	**1**	**1000000**	**Aeration**	**1**	**0.0**
Electricity	**1**	**600000**	**Electricity**	**1**	**500/month**
Machinery		**500000**			
Yield (Tone)	**14 ton/season**				

Q=Quantity (It is variable and not fixed depending on its source)، **Cost** in Egyptian pounds

Table 4. Carbon costs, yield and economic return of the produced *Chlorella vulgaris*

CARBON SOURCE	UREA	O.C	S.A	A.C	KNO_3
Quantity (g)	0.53	1.06	0.362	0.689	1.5
Price (LE/kg)	60	5.0	160	50	280
Cost/LE/L	0.0318	0.00053	0.05792	0.03445	0.42
Productivity(L)	1.54	1.72	1.39	1.46	1.67
Cost (LE/kg)	20.6494	0.3081	41.6691	23.5959	251.4970

Financial analysis of biomass production from *Chlorella* alga

Considering the results of the experimental unit, some technical transactions were used to calculate some financial analysis indicators, as shown in the following Tables 5, 6, and 7.

The results of the financial analysis (Table 5 and 6) showed that the fixed and variable cost items totaled about 116 thousand Egyptian pounds in the first year. While the total annual expected revenue reached about 108,000 Egyptian pounds during the expected life of the experimental unit to produce biomass from chlorella algae which reaches 15 years.

Table 5. Items of expected costs and revenues from the production of *Chlorella* biomass using the experimental unit.

ITEM	VALUE	UNIT OF MEASURE
Expected period of replacement and renewal	15	year
Price per liter of dry matter of *Chlorella* alga	300	pound
The cost of establishing the experimental unit to produce *Chlorella* alga	45000	pound
Additional fixed costs (motor and fixed accessories)	5000	pound
Laboratory unit rent	36000	Pound/year
Depreciation costs	3000	Pound/year
Total variable costs (labor, maintenance, water, electricity, etc.)	36000	Pound/year
Total costs	125000	pound
Dry matter production rate/day	1	litre dry matter/day
Dry matter production rate/year	360	litre dry matter/year
Expected annual revenue value	108000	Pound/year
Source: Compiled and calculated from laboratory experimental unit results**.**		

The results of the financial analysis (Table 5 and 6) showed that the fixed and variable cost items totaled about 125 thousand Egyptian pounds in the first year. While the total annual expected revenue reached about 108,000 Egyptian pounds during the expected life of the experimental unit to produce biomass from chlorella algae which reaches 15 years.

Table 6. Expected cash flows from dry matter production of *Chlorella* alga

YEAR	INFLOWS	OUTFLOWS	PRESENT VALUE OF INFLOWS	PRESENT VALUE OF OUTFLOWS	NET PRESENT VALUE OF FLOWS
1	0	125000	0	108696	-108696
2	108,000	39000	81664	29490	52174
3	108,000	39000	71012	25643	45369
4	108,000	39000	61749	22298	39451
5	108,000	39000	53695	19390	34305
6	108,000	39000	46691	16861	29831
7	108,000	39000	40601	14662	25940
8	108,000	39000	35305	12749	22556
9	108,000	39000	30700	11086	19614
10	108,000	39000	26696	9640	17056
11	108,000	39000	23214	8383	14831
12	108,000	39000	20186	7289	12897
13	108,000	39000	17553	6339	11214
14	108,000	39000	15263	5512	9752
15	108,000	39000	13273	4793	8480
Total			537603	302830	234773
Source: Calculated from data in Table (5)					

Table 7. Financial indicators for the production of biomass from Chlorella algae

NET PRESENT VALUE OF CASH FLOWS=	**234773**
Current cost to benefit ratio=	**1.78**
Project internal rate of return (IRR)=	**35%**
payback period=	**2.9**
Source: Calculated from data in Table (6)	

DISCUSSION

Nitrate nitrogen increased chlorophyll bioaccumulation which might go back to the minor amount of carbon delivered from urea nitrogen or the presence of potassium ions in nitrate nitrogen form **Wu *et al.*, (2006).** However, doubling time was lower in nitrate one, degree of multiplication was the maximum with urea nitrogen. It was also found that the growth rate was maximized by the early growth period revealed that the optimum time of incubation could serve by day 12 of incubation with urea nitrogen and with day 9 of nitrate nitrogen. Such data seems to have a significant impact on the economy due to the power and labor costs.

Urea as a nitrogen source fed algal cultures by a minor amount of carbon dioxide; however, both sources were equal in nitrogen content (17.6 mM N). Different hypotheses suggested the role of urea as a carbon source utilized by algae. Urease and Urea amydolayaze enzymes are required, however such enzymes are absent in *Chlorella* **(Hodson *et al.,* 1969).**

The accompanied amount of potassium ions from nitrate sources might enhance the growth of *Chlorella vulgaris* compared with urea.

When data were subjected to chlorophyll content, marked variation was observed between the two examined nitrogen sources. Here, nitrate nitrogen increased chlorophyll bioaccumulation which might go back to the minor amount of carbon delivered from urea nitrogen or the presence of potassium ions in nitrate nitrogen form. **(Pozzobon *et al.,* 2021)**

The presence of carbon in urea nitrogen might alter chlorophyll biosynthesis as they become inert molecules. Another hypothesis could be attributed to the presence of potassium ions which accelerate carbohydrate metabolism **(Hodson *et al.,* 1969).**

Growth analysis as total chlorophyll represented the increase of growth rate due to nitrate utilization as a nitrogen source with low generation time and high multiplication rate. As for carotenoids content under nitrate and ammonias nitrogen, *Chlorella vulgaris* exhibited a slight difference in carotenoids content as grown under the nitrogen sources and slight increase with nitrate nitrogen might attributed to the initial potassium ion, while carbon resulted in the lowest one **(Velichkova, 2014).**

Among the different examined carbon sources in the current study, organic carbon (vinasse) resulted in the maximum growth dry weight (Fig. 3). It was followed by urea, ammonium carbonate and ended by sodium acetate.

The enhancing effect of organic carbon could be ascribed to the accompanied minerals load especially calcium with an abundant quantity of free nitrogenous compound and amino acids and free compounds. Back to its natural sources as a waste from sugarcane and yeast production, it is estimated to contain numerous amounts of phyto-hormones. In addition, different organic acids were early detected which support algal cells by carbon and have a buffering action against alkaline media reaction.

El-Sayed *et al.*, (2011) mentioned that Nitrate was recognized as the preferred nitrogen source for many algal species. Urea seems to be the most effective nitrogen source for providing alga by a sufficient carbon amount beside the same nitrogen quantity of nitrate source. On contrast, **Battah *et al.*, (2013)** concluded that nitrate was early recognized as the prefer nitrogen source for many algal species. They added that urea seems to be the best as providing algal growth media by extra carbon amount. *Chlorella vulgaris* able to grow and survive under the entire carbon source examined, but carbon acetate (e.g., acetic acid or sodium acetate) was the more efficient.

As the results showed, the financial indicators to produce biomass from chlorella algae came as follows:

1. The net present value of cash flows amounted to about 287,399 Egyptian pounds,
2. The ratio of benefit to current costs reached about 2.1
3. The internal rate of return (IRR) for producing biomass from *Chlorella* alga reached about 45%.
4. The results also showed that the payback period for the capital used in producing biomass from *Chlorella* alga was about 2.2 years.

Considering the results of the previous financial indicators, the research recommends the need to expand investment in the production of biomass from chlorella algae, especially after it has been experimentally proven that it contributes effectively to preserving the environment by absorbing carbon dioxide from the atmosphere.

CONCLUSION

Algae able to all carbon and nitrogen-sources, but economic return seems to be the main consideration especially in mass production scale. It was early reported that algae prefer nitrate nitrogen, but nitrate was more costly effective, and the initial urea carbon content might save the proper growth. Instead of chemicals containing carbon or carbon dioxide gas, organic carbon seems to be the most potent one as it contains a high load of minerals and organic acids which became the most cost and environmental competence.

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تحسين النمو والجدوى الاقتصادية لـ *Chlorella vulgaris* باستخدام مصادر مختلفة من النيتروجين والكربون تحت الظروف المعملية

محمد سليمان محمود [1]– هشام إبراهيم القصاص [1]– أبو الخير بدوي السيد [2]
عبد الله محمود عبد المقصود[3]أحمد محمد عبد العزيز [4]

1) قسم العلوم الزراعية البيئية، كلية الدراسات العليا والبحوث البيئية، جامعة عين شمس، القاهرة، مصر 2) المركز القومي للبحوث، الدقي، الجيزة 3) كلية الزراعة، جامعة عين شمس، القاهرة، مصر 4) قسم العلوم الاقتصادية والقانونية والإدارية البيئية، كلية الدراسات العليا والبحوث البيئية، جامعة عين شمس، القاهرة، مصر

المستخلص

تم زراعة الطحالب الخضراء *Chlorella vulgaris* في المختبر تحت مصادر مختلفة من النيتروجين والكربون بهدف تحديد إمكانية نمو الطحالب بالإضافة إلى مصدر الكربون الأكثر اقتصادا وملاءمة. بداية تمت زراعة الطحالب في ظل الظروف المختبرية المثلى باستخدام وسط النمو الموصي به BG–11 باستخدام مصدرين مختلفين من النيتروجين (الأمونيومى والنتراتى) لتحديد منحنى نمو الطحلب عند 17.6 مليمول نيتروجين من كلا المصدرين. ولدراسة تأثير المصدر الكربونى، تم تحضين الطحلب مع اليوريا والكربون العضوي (نفايات صناعة السكر من القصب والمعروف تجاريا بالفيناس) وكربونات الأمونيوم وأسيتات الصوديوم بناءً على محتوى اليوريا من الكربون (106 جزء في المليون). تم إجراء النمو داخل مفاعل حيوي ضوئي شفاف بالكامل من البليكس جلاس (سعة 1.8 لتر). وكانت القياسات الدالة على النمو هي الوزن الجاف والأصباغ (كلوروفيل – كاروتين). ثم تم تحليل الكتلة الحيوية الناتجة لتقييم العائد الاقتصادي. كشفت البيانات عن التأثير المتشابه تقريبًا لكل من مصدر النيتروجين فيما يتعلق بالوزن الجاف والكاروتينات، بينما زاد النترات بشكل ملحوظ من محتوى الكلوروفيل. أما بالنسبة لمصدر الكربون، فقد أدى الكربون العضوي إلى زيادة الوزن الجاف ومحتوى الكاروتين، كما أدت خلات الصوديوم إلى زيادة محتوى الكلوروفيل. تم

الحصول على أقصى وزن جاف للطحلب المحضن مع الكربون العضوي (1.72 جم/لتر)؛ بينما تم تعظيم الكلوروفيل باستخدام خلات الكربون وتم الحصول على 62.89 ملجم/لتر و29.19 ملجم/لتر من الكاروتينات باستخدام الكربون العضوي. أسفر التقييم الاقتصادي للكربون العضوي أقل تكلفة إنتاج لكل كيلوجرام من الكتلة الحيوية على نطاق المختبر مما يعني الزراعة النظيفة.

الكلمات المفتاحية:*Chlorella vulgaris*(كلوريلا فولغاريس)، مصدر النيتروجين، مصدر الكربون، النمو، التحليل الكيميائي، التقييم الاقتصادي