EGYPTIAN ACADEMIC JOURNAL OF

# BIOLOGICAL SCIENCES

## PHYSIOLOGY & MOLECULARBIOLOGY

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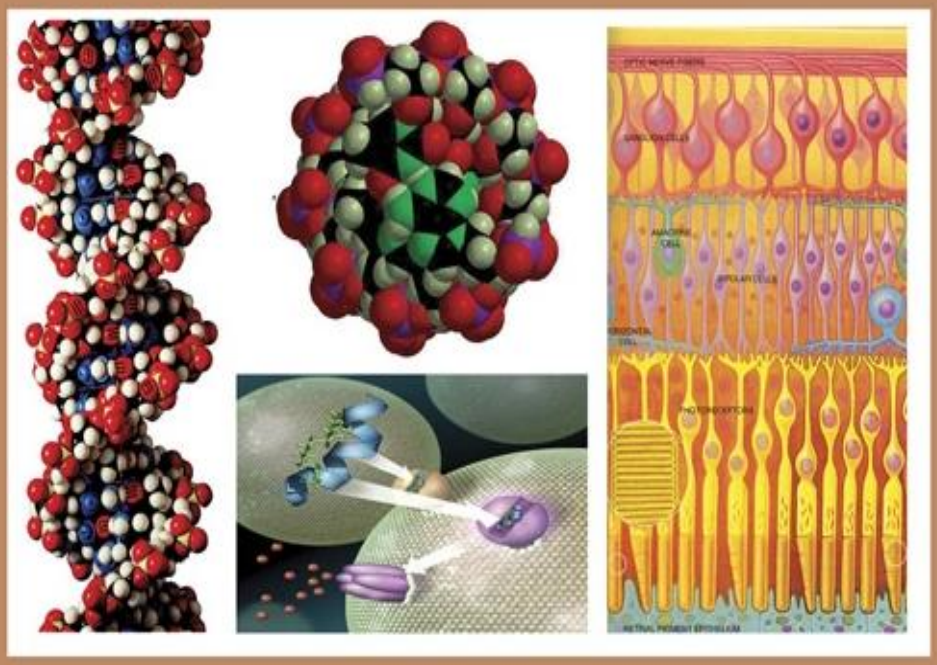

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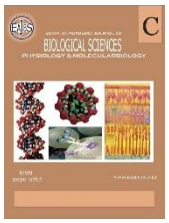

# Simplification of the Culture Medium and Action of The Bioreactor Material on The Stimulation of *Chlorella vulgaris* Production

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**ABSTRACT**

Cultivation *Chlorella vulgaris* still remains a challenge for scientists against the background of the cultural systems and the different cultural media deployed. In this context, our study aimed to a comparison of culture media BG11 known for its validity in the culture and new media GS1 and GS2 (rich in ammoniacal and nitric nitrogen) easy to prepare and used. Further culture parameters were involved such as photoperiod (16 h and 24 h) and the materials of the culture contents used in the bioreactor (glass bottles, plastic bottles in LDPE (low-density polyethylene) and plastic bottles in PET (polyethylene terephthalate)). The obtained results indicated that these parameters displayed a direct effect on the productivity of the culture and that their effect was mutual. However, for optimal production of *C.vulgaris* biomass, it is recommended to use a GS1 culture medium at a photoperiod of 24h, for a production of 50.6 x $10^6$ cells/ml, and fresh biomass of 0.35 g/L. It is however advised to respect the different types' rates of nitrogen in order to avoid cell division inhibition. Therefore, a concentration of 0.13 g/L of nitric nitrogen and 0.28 g/ L of ammoniacal nitrogen is required, with a low dose of urea nitrogen of 0.34g/ L.

The use of PET plastic in the bioreactor restricts the adhesion of the culture to the cylinders' walls, and with reduced loss rate, a homogeneous diffusion of the light in the totality of the cultures, and a lifetime of the bioreactor remains greater.

## INTRODUCTION

The species *Chlorella vulgaris* named *Chlorella*, is a unicellular freshwater microalga characterized by a considerable concentration of chlorophyll, edible for humans and animals. This species contains 60% of proteins useful for human food, zooplankton and fish breeding. The composition is of great interest due to its richness in several vitamins such as provitamin A, B1, B2, B3, B5, B6, B12, C, E and K. Furthermore, its richness in unsaturated essential fatty acids (omega-3 and omega-6), some minerals (iron, calcium, magnesium, zinc, potassium, etc.), and some enzymes and peptides (Larrondo Muguercia *et al*., 2020; Gael and Bruno, 2014);

This microalga includes further natural constituents that could ameliorate human and animal health. In addition, this species has shown detoxifying effects of heavy metals and other harmful compounds (lead, cadmium and naphthalene), and has shown also significantly reducing oxidative stress induced by these harmful compounds, with an increase in antioxidant activity (Rastoin, and Robin, 2016).

*Chlorella* is cultivated through various processes and cultural media. Its organic production currently motivates researchers and technologists who invest in improving productivity with the aim of achieving sustainable biomass and ensuring an ecologically and also economically viable bioresource.

The nutritional composition of *Chlorella vulgaris* is strongly influenced by the cultivation methods, the climatic conditions and the selected culture medium. Such as microalgae, culture procedures must be carried out with rigor and requirement (Gael and Bruno, 2014). Recent literature data have shown that microalgae, in the form of « organic » or « bio », are polluted with heavy metals or contain high levels of undesirable toxins. Most of these microalgae are cultivated in the open air under very profitable economic conditions (Kuo *et al*., 2021). However, microalgae cultivated in a closed and well-controlled environment, notably in glass tubes, are those providing the best guarantee of purity. Knowing that fermenters or bioreactors can also provide an identical guarantee of purity, but remain very expensive (Cathaud, 2021). The capacity of *Chlorella* to fix heavy metals leads to the use of a controlled closed device, avoiding any contact with air or water pollutants, and thus ensuring a superior quality of the final product (Gael and Bruno, 2014).

Several culture media have been tested and used in biomass production. Ilavarasi *et al.* (2011) summarized the effect of several cultural media on the quantitative and qualitative proliferation of *Chlorella vulgaris*. Among these studied cultural media, we can cite: the modified Hoagland medium (MHM), the basic Bold medium (BBM), the acidified basic Bold medium (ABBM), and the Half strength Chu 10 medium (HC10), and the BG11 medium (BGM). Other researchers such as Hadj-Romdhane *et al*. (2012) based their work on the use of Sueoka Autotrophic medium. Most of the results ensure that *C.vulgaris* requires a constant supply of Nitrogen in the form of NH4+, which should be 5 mmol/L as recommended by Van de Catsije *et al*. (2018).

*Chlorella* has also a unique characteristic that allows cells to reproduce rapidly. Scientists call this phenomenon the Chlorella Growth Factor (CGF). This is a complex that occurs during photosynthesis and allows each cell of chlorella to double in size approximately every 20 hours (Gael and Bruno, 2014). The chlorophyll content in *C.vulgaris* is stimulated by the high light intensity with a 12-hour photoperiod. This chlorophyll is detected through its color change, turning to dark green (Madhubalaji *et al*., 2020). Belkoura and Dauta (2008) found that the maximum growth rate is recorded at a temperature of 35°C, under an intensity of 300 $\mu E.m^{-2}. s^{-1}$ and continuous illumination. Thus, it is feasible to direct the cellular metabolism of this species towards the production of the desired metabolite, by

selecting the appropriate environmental conditions and the time of algal biomass harvest.

The cultivation of *Chlorella vulgaris* must be carried out with rigor and requirement since it is easily influenced by the cultivation conditions and the environment. For this purpose, our study aimed to address and develop a highly productive, and thus economically profitable, production process for Chlorella. Another factor determining the production was also considered in this study. It concerns the adherence of cells to the wall of the culture flask, thus reducing the harvest rate. Therefore, the selection of the culture medium should be the least complex in its composition, thus ensuring the ecological and economic framework of this production.

## MATERIALS AND METHODS

### 1-Multiplication of The *C.Vulgaris* Strain In Solid Culture Medium:

The Chlorella vulgaris strain was isolated from freshwater aquaculture, in March 2019 in Sidi-Bel-Abbes city, and identified by the Agro-Biotechnology and Sustainable Development (ABDD) research team of Nutrition Pathology Agro-Biotechnology and Health Laboratory at Djillali Liabès University of Sidi-Bel-Abbes. This strain was multiplied in Petri dishes in two different media (BG11, GS1), with five duplicates for each medium. Petri dishes were incubated at a photoperiod of 12 hours, a light intensity of 2000 µmol $m^{-1}$ $S^{-1}$, and at 25°C. After 15 days of growth, transplantation was performed from solid to liquid medium to guarantee its culture. Rigorous monitoring of the growth was based on microscopic observations, its proliferation and the color of the culture. The biomass in a liquid medium in the bioreactor was collected every 10 days by filtration through a sterilized 25 µm sieve, to be weighed in a fresh and dry state.

### 2-Optimisation of the Liquid Culture Medium:

Aiming to compare the effect of the culture media on the growth of *Chlorella*, BG11 (Stanier *et al*., 1971) was designated as the control culture medium as shown in Table 1. Another culture medium was prepared, inspired by the natural environment of the species, named GS (Table 2). This medium is composed of mineral water enriched with an organic liquid fertilizer for green plants. This fertilizer must meet the species requirements without causing mineral stress. Therefore, two concentrations of this fertilizer were tested in the mass propagation of the species: medium GS1 (0.5 ml fertilizer in 1 L water) and GS2 (1 ml fertilizer in 1L water). All culture media were sterilized by Ultras Violet light at 254 nm for 2 hours (Davaux *et al*., 2011).

**Table 1.** Composition of BG11 culture medium (Stanier *et al*., 1971).

| Mineral elements | BG11 g/L |
|---|---|
| $NaNO_3$ | 1.5 |
| $K_2HPO_4$ | 0.31 |
| $MgSO_4,7H_2O$ | 0.075 |
| $CaCl_2$, $2H_2O$ | 0.036 |
| Citric Acid | 0.006 |
| Ferriammonium Citrate | 0.006 |
| EDTA | 0.001 |
| $Na_2CO_3$ | 0.02 |
| $H_3BO_3$ | 2.86 |
| $MnCl_2$, $4H_2O$ | 1.81 |
| Zn $SO_4$, $7H_2O$ | 0.222 |
| $Na_2MoO_4$, $2H_2O$ | 0.390 |
| $CuSO_4$, $5H_2O$ | 0.079 |
| $CO(NO_3)_2$, $6H_2O$ | 0.0494 |
| pH | 7.1 |

**Table 2.** Mineral composition of GS culture medium Mineral water (Open Food Facts, 2021).

| Mineral composition | For 1000 ml | Organic fertilizer: NPK solution 7.5.6 | Mg/L |
|---|---|---|---|
| **Salt** | 0.4 g | Ammoniacal nitrogen $NH_4$ | 280 |
| **Sodium** | 0.16 g | Urea nitrogen $CO(NH_2)$ | 290 |
| **Bicarbonate** | 2.65mg | Nitric nitrogen $NO_3$ | 130 |
| **Potassium** | 21 mg | $P_2O_5$ | 500 |
| **Chloride** | 720 mg | $K_2O$ | 600 |
| **Calcium** | 810 mg | Copper | 0.2 |
| **Magnesium** | 240 mg | Iron | 2.5 |
| **Nitrite** | 0.2 mg | Manganese | 1.4 |
| **Nitrate** | 150 mg | Molybdenum | 0.1 |
| **Sulphate** | 530 mg | Zinc | 0.4 |
| | | EDTA | 0.01 |

**3-Experimental Set-Up:**

The device used is the controlled closed culture, according to the manufacturer recommendations research techniques of the company Alga-Labs (Lemir, 2014), and Pellerin *et al*. (2019). The closed system significantly reduces the risk of pollution and contamination of the culture, due to exposure to the open air. Three photobioreactors were studied, each of which had 20 bottles of specific materials, equally divided for BG11, GS1 and GS2 culture media. The first bioreactor was made of 1000 ml glass bottles and the second bioreactor was made of PET (polyethylene terephthalate) plastic bottles. However, the third one was made of LDPE (low-density polyethylene) plastic bottles. All systems were UV sterilized prior to usage. Agitation was provided by the rotary circuit created by air ejected sterilely through a filter into the culture. This device was exposed to a temperature of 27±2°C, a photoperiod of 18h and 24 h; and a light intensity of 2000 µmol $m^{-1}s^{-1}$. Continuous monitoring of the pH in the different culture media was maintained.

**4-Culture Strategy:**

- The first objective was to guarantee the purity of the production by culture on agar media (BG11 and GS1);
- The second objective was to study the production of *Chlorella vulgaris* biomass in a liquid medium under the effect of the parameters photoperiod (16h and 24h) and the culture media (BG11, GS1 and GS2). This was after the incorporation of a fraction of the agar cell culture in a liquid medium.
- The third objective was to determine the effect of cell adhesion to the walls of the bioreactor bottles on the production of fresh biomass of this species.

**5-Monitoring Parameters:**

- Perform weekly controls by microscopic observations of the cultures, and evaluate the purity of the cultures;
- Stationary phase determination of the cultures in the liquid phase for the photoperiods (16 h and 24 h) and for the culture media (BG11, GS1 and GS2) by measuring the number of cells by Hematimeter, triplicated;
- Determination of the fresh biomass as a function of the culture media and photoperiods;
- Statistical analysis by PCA of the results using Statistica 2012 software.

## RESULTS

*Chlorella vulgaris* isolated from aquaculture waters was identified at the level of Nutrition Pathology Agro-biotechnology and Health Laboratory (NUPABS), Djillali Liabès University of Sidi-Bel-Abbes (Algeria). That was one of the research project topics aiming to optimize the

cultivation and biomass production of *C.vulgaris*. The results obtained are summarized as follows:

**1-Multiplication of The Strain in Agar Medium:**

The seeding of this species in agar medium (BG11 and GS1) revealed the importance of the richness of the culture medium in trace elements in the speed of cell proliferation. After incubation, an increase in the GS1 culture was recorded from the third day of incubation and saturation of the Petrie dish after 15 days of cultivation (Fig. 1). Recurrent cultures, as summarized in Figure 2, showed that the species grew positively in BG11 medium only after seven incubation days, with saturation after 28 days of culture. Therefore, the use of GS1 medium favored a rapid cell multiplication of this species, and thus a better multiplication of the strain.

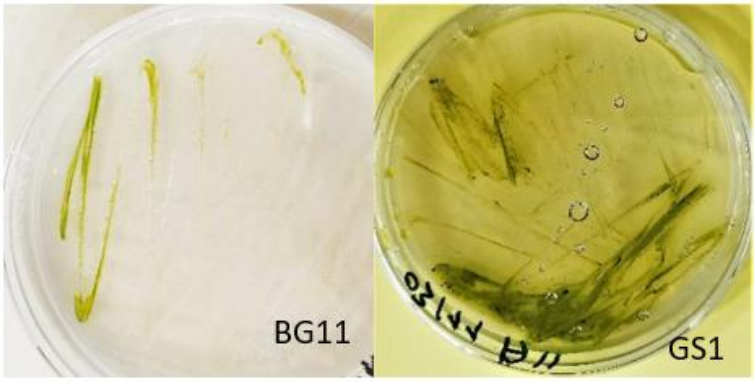


**Fig. 1.** Evolution of the culture in BG11 and GS1 after 9 days of incubation.

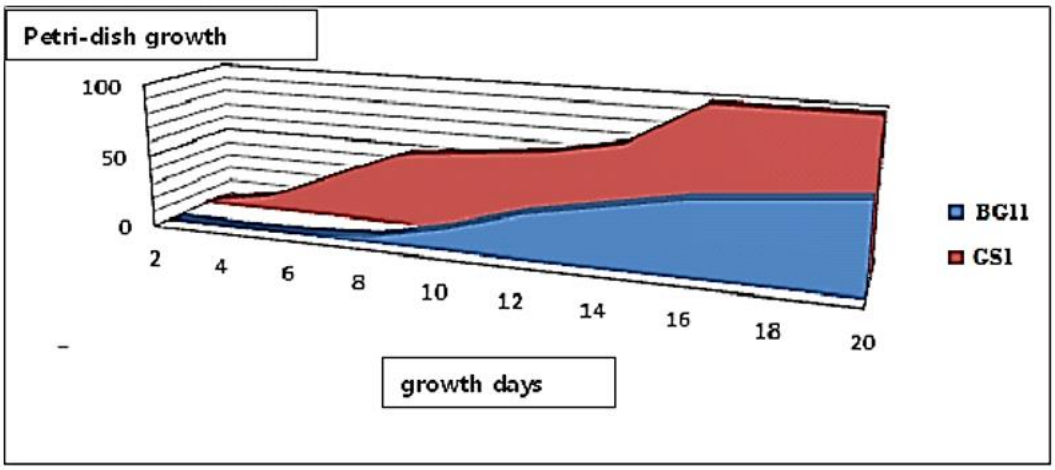


**Fig. 2.** Graphical representation of the saturation of culture dishes with *Chlorella vulgaris.*

**2-Purity of The Strain Culture:**

In the two culture-tested media (BG 11 and GS1), the binocular microscopy observation of the cells (Fig. 3 A) validated the strain purity with a spherical and typical shape of *Chlorella*. However, a difference in cell size was noticed in the two-culture media. Those in BG11 were larger than those in GS2. Average size of 5-6 µm was recorded for the cells in GS1 medium, whereas we recorded up to 10 µm for BG11 medium. This explains that cell division in GS1 medium was greater than that in BG11 medium (Figs. 3 A and C).

This difference in cell size in the two-culture media perfectly explains the higher cell division rate in GS1. This clarifies the slow proliferation of cells in the Petri dish of BG11 compared to GS1.

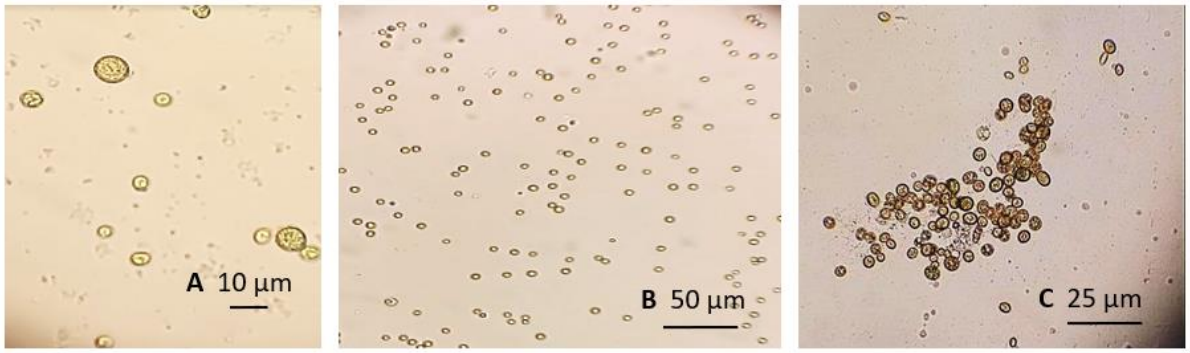


**Fig. 3.** *Chlorella vulgaris* in culture on agar medium.
**A.** *Chlorella vulgaris* form in BG11; **B.** culture on BG11 agar medium; **C.** culture on GS agar medium.

**3-Determination of The Stationary Phase of The Cultures:**

During the liquid phase culture of *Chlorella vulgaris*, the pH was comprised between 8 - 9.2. The determination of the stationary phase (exponential) was established in accordance with the culture media (BG11, GS1 and GS2), and in photoperiod (16 h and 24h). It was recorded that after 15 days of culture at 16 h photoperiod, in BG11 medium, the stationary phase was 13.04 x $10^6$ cells/ml. In contrast, in GS1 medium, the exponential phase was 24.2 x $10^6$ cells/ml. However, during the 24 h photoperiod, this phase was 26 x $10^6$ cells/ml for BG11 and 50.6 x $10^6$ cells/mL for GS1. The use of the GS2 culture medium (a medium very rich in nitrogen) limited the proliferation of *Chlorella* cells to a threshold of 10 x $10^6$ ± 2 cells/ml for both types of photoperiods.

**4-Fresh Biomass:**

The cells' proliferation in the culture media did not result in any residues indicating contamination. This confirms the efficiency of the bioreactor device, avoiding any source of contamination. Microscopic observation of the solution was performed before harvesting by filtration through a 20 µm sieve. Figure 4B reveals the formation of *Chlorella vulgaris* cells in gelatinous clusters, typical of the species, after 15 days of culture; thus, forming a pellet of a dark green color (Fig. 4A), and facilitating its harvesting. On the other hand, the dark green color of the culture (Figure 4A) confirms the richness of the cells in chlorophyll, and thus the validity of the favorable conditions of the culture.

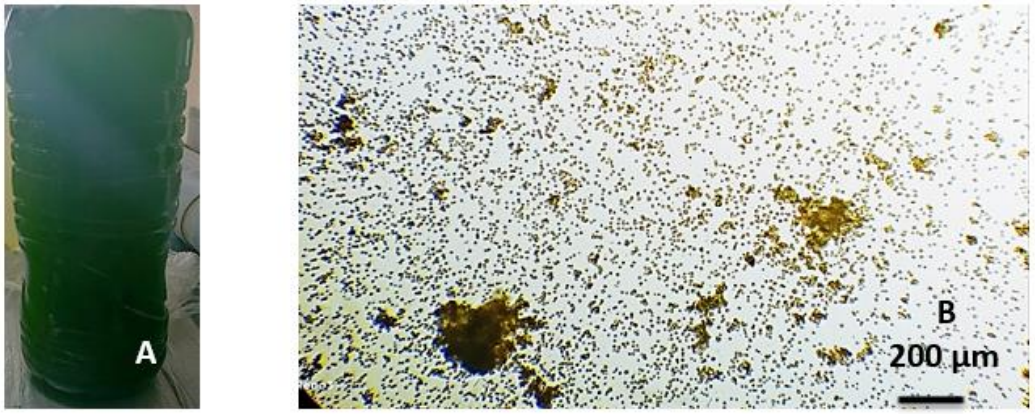


**Fig. 4.** Macroscopic (A) and microscopic (B) observation of the *Chlorella vulgaris* culture of 15 days growth.

The harvesting of the *Chlorella* biomass confirmed the importance of two parameters in the stimulation of cell division of the species studied. According to Figure 5 data, the composition of BG11 culture medium has a slight effect on cell proliferation; we found, for 16 hours of photoperiods, a change in harvest from 0.14 g/l of biomass in 10 days of growth to 0.15 g/L for 15 days of culture. A slight increase in biomass was noticed for the other culture media under the same culture conditions. However, a continuation of the photoperiod up to 24 h stimulates the biomass productivity, reaching a maximum rate of 0.35 g/L in GS1 medium after 15 days of culture.

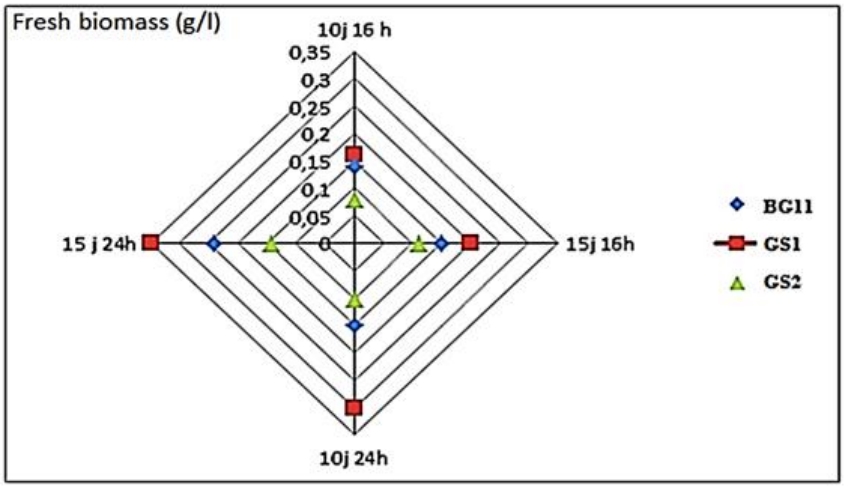


**Fig. 5.** Harvested biomass of *Chlorella vulgaris* as a function of growth time (d: days) and photoperiod (h: hours) for the BG11 culture media GS1 and GS2.

It is therefore justified, according to Figure 6 data, that the presence of nitric and ammoniacal nitrogen in GS1 medium at a concentration of 0.13 g/L and 0.28 g/L respectively; associated with a low concentration of urea nitrogen, strongly stimulates cell division in a 24 h photoperiod. On contrary, their high concentration in GS1 medium significantly delayed cell division of *Chlorella vulgaris*. This explains the low productivity of the biomass in BG11 medium, which has a very high ureic nitrogen concentration of 1.54 g/L.

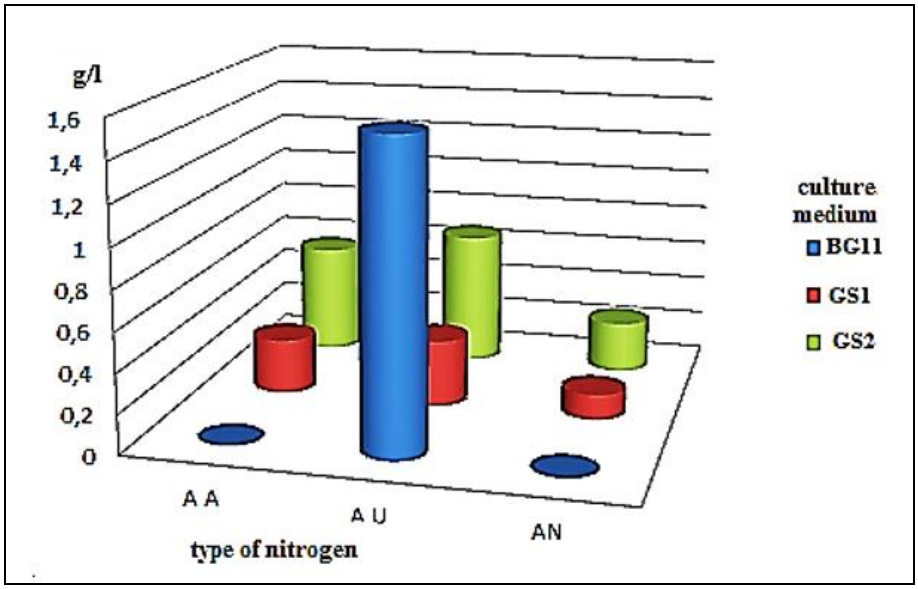


**Fig. 6.** The concentration of different types of nitrogen in culture media.
AA: Ammoniacal Nitrogen; AU: Ureic Nitrogen; AN: Nitric Nitrogen

**5-Adherence of The Species to The Materials Used:**

In this study, three types of bottle materials (glass, PET plastic and LDPE plastic) were utilized in the culture of *C.vulgaris* at a photoperiod of 16 h and 24 h. The agitation of the culture in the contents was ensured by the movement of air bubbles during the oxygenation of the culture medium. At harvest, the bottles were shaken to remove the cultures as much as possible before being filtered. However, a film of culture forms on the walls of the bottles, the thickness of which differs according to the used material. For all the culture media, a significant cell adherence was recorded on 80% of the glass walls; this leaves a very important margin of loss in production estimated at 8%. An identical result was recorded for LDPE plastic bottles, where the adherence to the culture encompassed nearly 60% of the bottle, and thus an estimated loss of 6% of the overall production. However, the use of PET bottles revealed slight culture adhesion (25%) were shaking the bottle was sufficient to remove the culture from the walls, prompting the reuse of PET bottles in the bioreactor. The low adhesion rate of the microalgae to the PET plastic material provides a low loss in production estimated at 1%, from which a maximum harvest of Chlorella biomass was recorded.

**6-Statistical Analysis:**

A statistical study by PCA (principal component analysis) of the results obtained from the multiplication of *Chlorella vulgaris* was applied, according to the biomass, depending on the culture time and photoperiod (10d 16 h, 15d 16h, 10d 24h, 15d 24h), of the compounds in different nitrogen (AA (Ammoniacal Nitrogen), AU (Urea Nitrogen) and AN (Nitric Nitrogen)) of the culture media (BG11, GS1, GS2).

The analysis was then performed by a matrix resulting from the correlation crossing between the variables (10d 16 h, 15d 16 h, 10d 24h, 15d 24h, AA, AU and AN) and the Cell Multiplication Stimulation Factors (F1) and Culture Media (F2) (GS2, GS 1 and BG11). These results are reported in three independent groups (Gr1, Gr2, Gr3) (Figs. 7 and 8). There was a strong positive correlation between the variables GS1, AN and AA with the axis1 of the biomass productivity component, with a value close to 1, and with a strong contribution (0.798237). It is a growing medium characterized by an average presence of different types of mineral salts and nitrogen (AN and AA). This confirms the validity of the observations described previously, and that these two types of nitrogen are very

stimulating for the multiplication of *Chlorella vulgaris*.

The growth time and photoperiod were negatively correlated with this same axis, forming another group (Gr3) with the GS2 culture medium. This group is therefore the opposite of the Gr1 group. It was strongly correlated with the following variables with their contributions: 10d 16 h (-0.992841), 15d 16h (-0.893899), 10d 24h (-0.945525) and 15d 24h (-0.964220). This explains that it is a culture medium characterized by a strong presence of the different types of nitrogen, prompting a longer time with a long photoperiod so that there can be stimulation of biomass productivity.

However, Gr2 group encompasses the AU variables strongly correlated with BG11 environment, with a contribution of -0.122014. BG11 medium was characterized by the absence of ammonia (AA) and nitrate (AN) nitrogen, with a considerable amount of urea nitrogen (AU). This constitutes a group negatively close to "0" of the productivity axis "1".

The hierarchical ascending classification (HAC), as shown in Figure 8, identified and confirmed the three groups of the studied variables and highlighted them by the PCA (Fig. 7). Variable 10 d 16h is closest to "0" on the vertical axis (production time), but at least 1 on axis 1. This means that the stimulation of biomass production does not depend on time or photoperiod, but depends much more on the presence of the three types of nitrogen (AA, AU and AN) at average doses. However, photoperiod and cultivation time are involved in increasing biomass over time.

At the factorial level, F1 axis provides the most information in the PCA (69.97% inertia), compared to F2 axis (30.03%), which opposes Gr1 group to the two groups Gr2 and Gr3. This analysis revealed the conformity of this study with the observations disclosed previously; notably the importance of the presence of ammonia nitrogen and nitrate nitrogen for very favorable productivity of the biomass. It also showed that the factors cultivation time and photoperiod time display no positive effect on biomass, in case of a low presence of these two types of nitrogen (AA and AN).

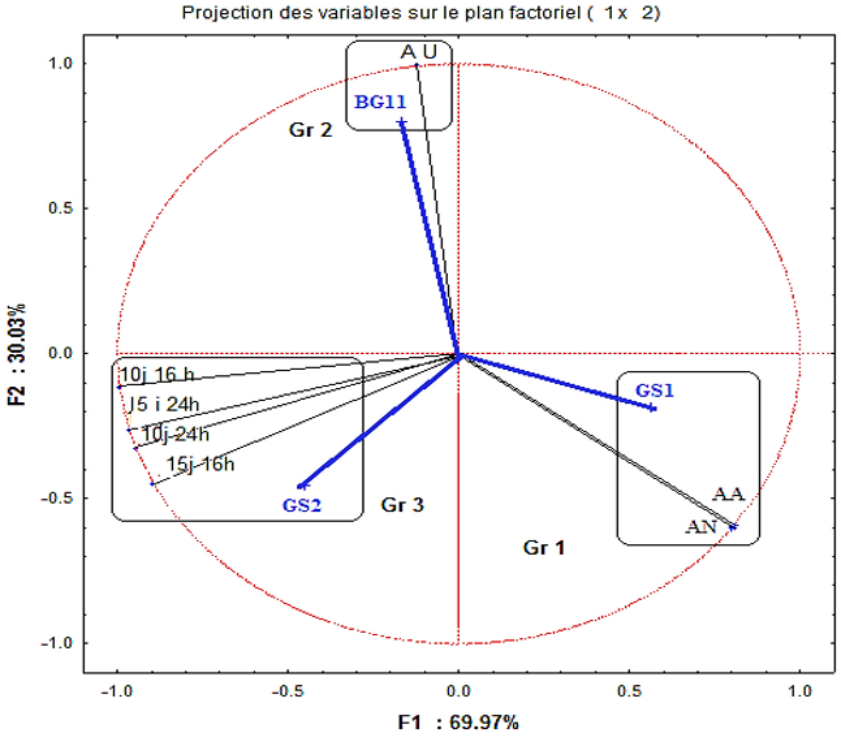


**Fig. 7.** Graphical representation of the PCA (correlation circle, plane 1-2).
Growth time (J) photoperiod (h), Gr: group; F1 factor 1 (biomass productivity boost); F2. factor 2 (crop time); AA. ammonia nitrogen; AU. urea nitrogen; AN. nitrate nitrogen.

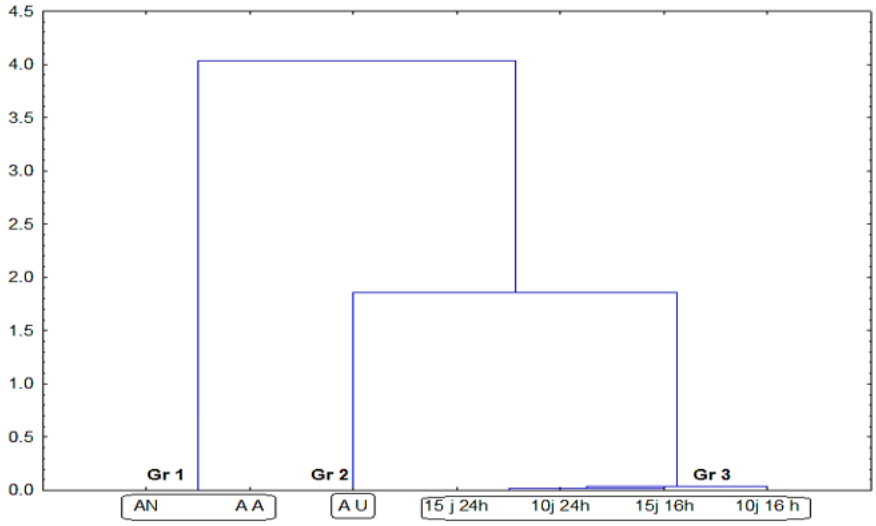


**Fig. 8**. Graphical representation of AMP.

## DISCUSSION

Microalgae, in particular *Chlorella vulgaris*, seem to be one of the alternatives in the production of biomass useful to develop bioproducts, such as biofertilizers, biofuel, or para-pharmaceuticals. In this study, *Chlorella vulgaris* was optimized for productivity, taking into account growth parameters and factors. This objective has interested several scientists and scholars, leading to debates on their optimal cultural conditions (Pellerin *et al*., 2019).

One of the major issues encountered by researchers is the selection of culture medium, which is generally made up of numerous chemicals that are not economically viable. Following preliminary tests and on natural environments favorable to this species, GS1 medium was tested for its purification and multiplication in Petri dishes and in the bioreactor device. The results obtained illustrate the efficiency of the junction between mineral water and biological fertilizers for the production of green microalgae, in particular *Chlorella vulgaris*. The use of this type of cultural medium is a promising and economically profitable alternative. The ease of preparation will allow for further development of biomass production and thus more microalgae-based products. In this area, it is essential to determine the main components of a culture medium for better productivity. De Carvalho *et al*. (2019) and Lemir (2014) have been invested in comparing different culture media by specifying the determining elements that stimulate cell division in these media. Other researchers focused on facilitating culture by presenting a recyclable culture medium. In all cases, it was noted by all these authors that nitrogen concentration constitutes a factor in the development of *C.vulgaris* cultures. In our study, the culture media was tested to confirm very productive biomass with the presence of all three types of nitrogen (Nitrates, Nitrites and Ammonia). Taufikurahman *et al.* (2020) pointed out in their research that *Chlorella vulgaris* uses ammonia and nitrate nitrogen to produce proteins. These researchers confirm the importance of the presence of ammoniacal nitrogen in the culture medium, which represents the principal source for *C.vulgaris*, since it is directly absorbed by the cells without going through its initial transformation (Taufikurahman *et al*., 2020). This agrees with the results obtained with GS1 culture medium, with similar biomass. However, a higher dose of ammonia induced a slowdown in cell proliferation and subsequently reduces biomass. This is consistent with the research results of Blanco et al. who explained that this species is also inhibited by a high presence of ammoniacal nitrate, leading to a strong stagnation of

growth and therefore low biomass productivity.

The use of the closed system for its large-scale production ensures its quality, but low biomass productivity. Xia & Murphy (2016) reported that in large-scale production, nutrient requirements can account for up to 50% of the total cost of biomass production. Its high costs have triggered efforts to find a cheaper and economically feasible approach to its cultivation. Studies by Nasir *et al*. (2015) and Luo *et al.* (2016) reported that microalgae can be grown using wastewater and aquaculture water instead of the expensive synthetic medium, as it contains all nutrients required for algal growth. In our study, replacing the culture medium with mineral water enriched with NPK fertilizers 7, 5, 6 strongly stimulated the growth of microalgae and thus reduced the cost of the culture. It is therefore approved through the results that GS1 culture medium is the most suitable for optimal biomass productivity, at a reduced cost.

Generally, the use of tubular bioreactors is highly recommended for quality production. Lucchetti (2015) illustrated in his works that the junction between the cylindrical system and the rotational movement of the culture medium favors a homogenization of the culture, through a good distribution of light in the system. In our study, the results showed that additionally to these parameters, the type of material of the bioreactor cylinders could have a direct influence on the loss rates of the large-scale culture. Over time, a thin layer of the culture settles and adheres to the cylinder walls, leading to a decrease in microalgae productivity. This is consistent with the observations made by Sirmerova *et al*. (2013). This adherent layer of microalgae increases with time and decreases the light distribution. Carvalho *et al*. (2011) explained that microalgae, including *C.vulgaris*, adhere to the walls to get the maximum amount of light. This theory remains reasonable to ensure its photosynthesis. A further factor determining the adhesion of microalgae to walls is the electrostatically attractive interactions between algae and solid surfaces as shown by Xu *et al*. (2011). It is well-established that the surface charge of *C.vulgaris*, at a pH close to neutrality, is negative (Hadjoudja *et al*., 2010). In our study, PET plastic is considered antistatic (INRS, 2020), and therefore *C.vulgaris* cells do not adhere to the PET walls. For LDPE plastic, the material is electrostatically stable but can become charged (SLOVNAFT, 2016). This could explain the attractive effect of *C.vulgaris* cells on the walls. However, the problem is when using glass matter is considered the best light scatterer. Glass has a static charge accumulated on the surface which eventually exerts an electrical force and therefore attracts elements with no or very little charge, i.e. static electricity (Vega, 2015). It is therefore the most attractive material for the culture of *C.vulgaris*; thus inducing the formation of a culture film on the glass wall, and thus a decrease in the profitability of the production of biomass. The study of the mechanism of microalgae from an industrial and commercial point of view is therefore of great importance for both process productivity and hygiene.

## Conclusion

*Chlorella vulgaris* is a species that is used extensively in all areas, especially for health purposes due to its high chlorophyll and protein contents. The cultivation of this species remains a major issue for biomass production and it is necessary to review and assess its cultivation technique in order to minimize its cost. In this context, two parameters were targeted: the culture medium and the bioreactor system. The selected species in the current investigation, *Chlorella vulgaris*, was collected, isolated and identified at the NUPABS research laboratory (Sidi-Bel-Abbes, Algeria).

The use of GS1 culture medium is appropriately adapted to the *Chlorella vulgaris* species by its richness in mineral elements (from mineral water), and by the different nitrogen compounds (by the liquid fertilizer NPK 7, 5, 6). This culture medium,

whether agar or not, remains the most appropriate for the production of the biomass for this species. An early proliferation of its culture was recorded in this agar medium from the third incubation day. This is twice as fast as the BG11 medium.

The photoperiod regulation is essential for the optimization of productivity. The results obtained highlight the importance of the 24-hour photoperiod, which doubles the cell multiplication from 24.2 x $10^6$ cells/ml in 16 hours of photoperiod to 50.6 x $10^6$ cells/ml in 24 hours of photoperiod in GS1 medium. On the other hand, it is not sufficient to increase the photoperiod to obtain maximum biomass production but to meet the nitrogen demand of the cultured cells. Therefore, a concentration of 0.13 g/L nitrate nitrogen and 0.28 g/L ammonia nitrogen, with a low urea nitrogen concentration of 0.34 g/L strongly stimulates cell division in a 24 h photoperiod. Under these conditions, we recorded an optimal fresh biomass production of 0.35 g/L in GS1 medium and 0.2 g/L in BG11 medium.

Another challenging element to consider is to design a bioreactor with a material that prevents the formation of biofilm on the walls, and thus, limits the losses in culture for maximum homogeneity in the distribution of light intensity. For this purpose, three types of materials were tested in the bioreactor: glass, LDPE plastic and PET plastic bottles. It was noticed that the microalgae adhered poorly to the PET bottles, resulting in a low biomass harvesting loss of 2.5%. On the opposite, the glass bottles were the most attractive to the culture cells, creating a fairly thick layer of the species, and thus covering almost the entire surface of the bottles; resulting in a large loss in harvested biomass estimated at 8%. This may not appear important, but in a large-scale culture, the loss could be highly considered.

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## REFERENCES

Belkoura, M. and Dauta, A. (2008). Interaction lumière-température et influence de la photopériode sur le taux de croissance de *Chlorella sorokiniana* Shih et Kraus. Biology Annales De Limnologie. *International Journal of Limnology*. Volume 28, issue 2. Pages 101-107.

Carvalho, A.P. ; Silva, S.O. ; Baptista, J. M. and Malcata, X. F. (2011). Light requirements in microalgal photobioreactors: an overview of biophotonic aspects. *PubMed Applied Microbiology and Biotechnology.* Volume 89, issue5. Pages 1275–1288.

Cathaud, M. (2021). Tout savoir sur la Chlorella: Rencontre avec Muriel Cathaud. In Web Page: *Journal de Santé, Conseils et Astuces. Nutrition, Femininbio (ED-54935). France.* https://www.femininbio. com/ sante/conseils-et-astuces/tout-savoir-sur-la-chlorella-rencontre-avec- muriel-cathaud-54935.

De Carvalho, J.C.; Bittencourt, E.; Ferrari, S.L.; Tessari, A. and Soccol, C. R. (2019). Culture media for mass production of microalgae, Chapter 2. In Culture media for mass production of microalgae Biofuels from Algae. *Elsevier, Science*

*Direct* ED (Second Edition). *Journals & Books Biomass, Biofuels, Biochemicals, France.* Pages 33-50.

Davaux, F.; Houbart, C. and Carrère, M. (2011). Les UV-C une alternative à la stabilisation microbiologique des moûts et des vins. *Revue des Oenologues et des Techniques Vitivinicoles et Oenologiques: magazine trimestriel d'information professionnelle,* Volume 38, issue 141. Pages 32-35.

Gael, B. and Bruno, J. B. (2014). Microalgues : de petits végétaux aux grandes promesses !. *Biofutur,. Elsevier* (France-Editions) *Scientifiques Medicales Elsevier*. Volum 12, issue 360. Pages 28-31.

Hadj-Romdhane, F.; Jaouen, P.; Pruvost, J.; Grizeau, D.; Vooren, G. V. and Bourseau, P. (2012). Development and validation of a minimal growth medium for recycling *Chlorella vulgaris* culture. *HAL Open Science*. Volume 85, Issue 123. ID: hal-02534145. 10 p.

Hadjoudja, S.; Deluchat, V. and Baudu, M. (2010). Cell surface characterisation of Microcystis aeruginosa and *Chlorella vulgaris*. *Journal of Colloid and Interface Science*, 342:293–299. In Sirmerova, M.; Prochazkova, G.; Siristova, L.; Kolska, Z. and Branyik, T. (2013). Adhesion of *Chlorella vulgaris* to solid surfaces, as mediated by physicochemical interactions. *Journal of Applied Phycology*. Volume 25, issue 6 DOI 10.1007/s10811-013-0015. Pages 1687–1695.

Ilavarasi, A.; Mubarakali, D.; Praveenkumar, R.; Baldev, E. and Thajuddin, N. (2011). Optimization of various grouth media to freshwater microalgae for biomass production. *Biotechnology,* volume 10, issue 6. Pages 540-545.

INRS. (2020). Plastiques, Risque et Analyse Therm IQue : Polyéthylène téréphtalate PET. Web Site : Base de données. Santé et Sécurité au Travail. *Numéro de Juillet-Août. Lyon, France.* https://www.inrs. fr/dms/ plastiques/Polymere Plastiques/ plastiques_ polymere_ 15-6/polymere_pet.pdf.

Kuo, C. M.; Sun, Y. L.; Lin, C. H.; Wu, H. T. and Lin, C. S. (2021). Cultivation and Biorefinery of Microalgae (*Chlorella* sp.) for Producing Biofuels and Other Byproducts. *A Review Sustainability,* Volume 13, issue 13480. 30 p.

Larrondo Muguercia, H.DL.M.; León Pérez, D.O.; Ricardo, G. and Rojas, G. (2020). Ciencias clínicas y patológicas artículo original Evaluación del estado nutricional en acientes Críticos mediante dos Índices de riesgo nutricional Assessment of nutritional status in critical ill patients by means of two Nutritional Risk Indexes. *Revista Habanera de Ciencias Médicas*, Volume 19, issue 4. ISSN 1729-519X. 14 p.

Lemir, P.O. (2014). Analyse théorique du projet Vertech I de Victoriaville. Thèse de doctorat, Centre universitaire en Environnement et développement durable, Université Sherbrooke, Québec, Canada. 265 p.

Lucchetti, A. (2015). Modélisation et conception d'un système de culture de microalgues. Génie des procédés. Thèse de doctorat, Ecole Nationale Supérieure des Mines de Paris, Français. Ffnnt: Enmp0048ff. Fftel-01141894f. École doctorale n° 432: Sciences des Métiers de l'Ingénieur.

Luo, L.; He, H.; Yang, C.; Wen, S.; Zeng, G.; Wu, M.; Zhou, Z. and Lou, W. (2016). Nutrient removal and lipid production by Coelastrella sp. in anaerobically and aerobically treated swine wastewater. *Bioresour Technology,* Volume 216. Pages

135–141.

Madhubalaji, C. K.; Sarat-Chandra, T.; Chauhan,V. S.; Sarada, R. and Sandeep, N. M. (2020). *Chlorella vulgaris* cultivation in airlift photobioreactor with transparent draft tube: effect of hydrodynamics, light and carbon dioxide on biochemical profile particularly ω-6/ω-3 fatty acid ratio. *Journal of Food Science and Technology*, volume 57. Pages 866–876.

Nasir, N. M.; Bakar, N. S. A.; Lananan, F.; Abdul Hamid, S. H.; Lam, S. S. and Jusoh, A. (2015). Treatment of African catfish, Clarias gariepinus wastewater utilizing phytoremediation of microalgae, *Chlorella* sp. with *Aspergillus niger* bio-harvesting. *Bioresour Technology*, Volume 190. Pages 492–498.

Open Food Facts. (2021). Eau minérale Ifri - 0.5 L. Web Site : Une base de données collaborative, libre et ouverte des produits alimentaires du monde entier. Openfoodfacts-contributors, CET. https://world-fr.openfoodfacts.org/produit/ 6130093010045/eau-minerale-ifri.

Pellerin,R. ;; Qureshi, H.; Thiriez, F.; Van de Catsije, M. (2019). Etude de conditions ideales pour le developpement de *Chlorella vulgaris*, et la fabrication d'ethanol. Rapport presenté dans le cadre du Projet d'Apprentissage par projet. Institut Villebon Georges Charpak Paris Saclay et Paris Sud. 54 p.

Rastoin, J.L. and Robin, K. (2016). Le secteur des microalgues en Méditerranée Perspectives et contribution au développement durable. L'Institut de prospective économique du monde méditerranéen, IPEMED (ED). France. 94p.

Sirmerova, M.; Prochazkova, G.; Siristova, L.; Kolska, Z.; Branyik, T. (2013). Adhesion of *Chlorella vulgaris* to solid surfaces, as mediated by physicochemical interactions. *Journal of Applied Phycology*, DOI 10.1007/s10811-013-0015. Numéro 25, issue 6. Pages 1687–1695.

Slovnaft. (2015). Polyethylene basse densite bralen fiche de données de sécurité conforme au Règlement (CE). Numéro 830. Page 5.

Stanier, R. Y.; Kunisawa, R.; Mandel, M. and Cohen-Bazire, G. (1971). Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriological Reviews*. Volume 35, issue 2. Pages 171-200.

Taufikurahman, T.; Ardiansyah, M. A.; Astutiningsih, N. T.; Suyono, E. A. (2020). Cultivation of *Chlorella vulgaris* in Anaerobically Digested Dairy Manure, Wastewater (ADDMW) for Protein and Chlorophyll Production. *Sains Malaysiana*, Volume 49, issue 9. Pages 2035-2042.

Van de Catsije, M.; Pellerin, R.; Qureshi, H.; Thiriez, F. (2018). Effet de la concentration en azote sur le devellopement d'une culture de *C.vulgaris*, et de la privation d'azote sur la production de glucide par la cellule. DOI: 10.13140/RG.2.2. 34811. 16165. Project: Apprentissage par Projet 2 - Institut Villebon Georges Charpak.

Vega, P. (2015). Electricité statique. Colloque Mauricie 19e Carrefour en Santé et sécurité du travail. Version Web. Association sectorielle Fabrication d'équipement de transport et de machines. 3565, Jarry Est, Bureau 202 Montréal (QC) H1Z 4K6 https:// asfetm. com/wp-content/uploads/ 2013 /11/ Drummondville-4-novembre-2015-Electricite-statique-Web.pdf.

Xia, A. and Murphy, D. (2016). Microalgal Cultivation in Treating Liquid Digestate from Biogas Systems.

*Trends in Biotechnology*, Volume 34, Issue 4. Pages 264-275.

Xu, L.; Guo, C.; Wang, F.; Zheng, S. and Liu, C. Z. (2011). A simple and rapid harvesting method for microalgae by in situ magnetic separation. *Bioresour Technol*. Volume 102. Pages 10047–10051. In Sirmerova, M.; Prochazkova, G.; Siristova, L.; Kolska, Z. and Branyik, T. (2013). Adhesion of *Chlorella vulgaris* to solid surfaces, as mediated by physicochemical interactions. *Journal of Applied Phycology*, DOI 10.1007/s10811 - 013-0015. Volume 25, issue 6. Pages 1687–1695.