Available online at www.sciencedirect.com
ScienceDirect
Energy Procedia 74 (2015) 1402 – 1409
Energy Procedia

International Conference on Technologies and Materials for Renewable Energy, Environment and Sustainability, TMREES15

Growth and Nitrogen Removal Efficiency as Protein Content of Spirulina from Tertiary Municipal Wastewater in Ouargla

(Algerian Bas-Sahara)

Afaf DJAGHOUBI[a,b*], Mustapha DADDI BOUHOUN[b], Samia HADJ SAID[a], Ali SAGGAÏ[c], Sarah SOBTI[d], Belhadj HAMDI AISSA[d]

[a]*Univ Ouargla, Lab. The Underground Petroleum Tanks, Gaziers and Aquifers,Ouargla 30 000, Algeria*
[b]*Univ Ouargla, Lab. Ecosystem Protection in Arid and Semi Arid zones, Ouargla 30 000, Algeria.*
[c]*Univ Ouargla, Department of Agronomic Sciences, Faculty of Natural and Life Sciences, Ouargla 30 000, Algeria*
[d]*Univ Ouargla, Lab. Biogeochemistry of desert environments,Ouargla 30 000, Algeria*

Abstract

The green alga Spirulina, *Arthrospira platensis* was grown under shelter condition. The aim of this work is to find an approach for biologically recycling wastewater by Spirulina (strain of Tamanrasset) in area of Ouargla. The efficiency of Spirulina on the removal of N by evaluating protein content and biomass productivity were calculated, in comparative study, using two different culture mediums, conventional water medium CWM and Wastewater medium WWM. The use of wastewater was evaluated as a low-cost medium. The Good growth was obtained in wastewater medium; the maximum cell concentration and protein content achieved in WWM medium were 2.92±0.14 g L^{-1} and 24.5±2.83 % respectively. By adding 10% to the protein content, the cell content of nitrogen organic removal could estimate. It appears that the integration of Spirulina with wastewater is a promising way of simultaneously treating wastewater in our region and producing a biomass which could be incorporated into animal feeding.

Peer-review under responsibility of the Euro-Mediterranean Institute for Sustainable Development (EUMISD)
Keywords: Wastewte; Spirulina; growth; protein; Ouargla; Algeria.

1. Introduction

The reuse of treated wastewater is a need that has many interests, both on environmental and economic plans. Ouargla experienced in recent years a rise problem in the water table, this is due to the anarchic multiplication of

Peer-review under responsibility of the Euro-Mediterranean Institute for Sustainable Development (EUMISD)
doi:10.1016/j.egypro.2015.07.786

drilling, the poor drainage of agricultural water and wastewater management [1,2,3,4]. The WWTP (wastewater treatment plant) of this oasis discharge directly the tertiary purified effluents into Sebkha Safioune. Enhancing these effluents saves the groundwater resource in this oasis, only one available [3].

The use of microalgae in wastewater treatment was first proposed by Oswald and Gotaas (1957) [5], and has received much attention in recent decades [6,7,8,9,10,11]. Extensive research has been conducted to explore the feasibility of using microalgae to treat wastewater, especially for the removal of nitrogen, phosphorus [12,13]. The relationships between environmental characterization of Ouargla and cultural factors , which govern productivity in outdoor cultures, is in connection with growth yield and efficiency conditions, this environment are extremely productive because of high ambient temperatures, high light irradiance and unlimited supply of CO_2.

Microalgae are photosynthetic microorganisms which use energy from the sun to grow, consuming inorganic nutrients and CO_2. They accumulate organic matter in the form of proteins, lipids, carbohydrates, hydrocarbons and other small molecules and pigments. Micro algal biomass has been studied and used for human and animal nutrition and for producing substances such as fatty acids, b-carotene, astaxanthin and phycocyanin [14,15,16].

The microalgae, *A.platensis* is founded in Algeria, in guelta (point of mountain water) in Tamanrasset, that resembles to the Paracas strain sp [17,18]. *A.fusiformis* is also found in some lakes of the northern Algerian Sahara having saline waters (1–8%) and pH levels from 7 to 9[17.19]. These Cyanobacteria require higher amount of nutrients (N, P) for their growth, therefore, wastewaters rich in nutrients can be used as growth medium for their cultivation and indirectly they sequester these nutrients from wastewater [15,20]; simultaneously, the cultivation of microalgae can provide a useful source of biomass. It has been estimated that about 30% of the current world algal production is sold for animal feed application, aquaculture and poultry feed [21]. Furthermore, nutrients are not only removed from the wastewater, but can also be captured and returned to the terrestrial environment as agricultural fertilizer [22]

Several studies on growth and nutrient removal from wastewater by microalgae was carried on Chlorella [23,24]; *Scenedesmus sp.* [25,26]; Spirulina [22,27,28, 29], These studies have evaluated only the quality of the final effluent, and few have determined the nitrogen incorporation efficiency as protein. Therefore, in this present preliminary comparative study, we studied the kinetics of growth of Spirulina on tertiary municipal wastewater and conventional water medium and nitrogen removal as protein content under shelter condition.

2. Materials and Methods

2.1. Sampling of wastewater

Samples were collected from the tertiary municipal effluents of the WWTP (wastewater treatment plant) located in Ouargla, Algeria. The main element of the treatment system is the biological reactor, where the natural microorganisms remove the organic material by the aerated lagoon process. Thus, this procedure was found to be ineffective towards trace element removal. The physicochemical analyzes were performed at laboratory of National Agency Water Resources Ouargla, and presented in Table 1.

2.2 *Growth medium and cultivation conditions*

The Spirulina strain used in this study was previously obtained from guelta Atakor in Tamanrasset (Algeria) [30]. *S.platensis* was grown in two different medium. Wastewater medium (WWM), It consists of 16g L^{-1} natron. The addition of natron was necessary in order to adjust the pH of the effluent. The second medium, conventional water medium (CWM), was prepared by Albian water (Table. 1), this medium (CWM) was produced by adding: 0.2 g $L^{-1}$of $(NH_4)^2$ SO_4 , 0.05 g L^{-1} FeSO4, 0.1 g L^{-1} K_2SO_2 and 16 g L^{-1} natron.

The cultures were grown in plastic flasks with volume of 1.5L under shelter at the agricultural exploitation on faculty of natural and life sciences, Ouargla University, with an initial cell density of 0.67g L^{-1} in all the groups, bubbled with air, no temperature regulation was provided or autoclaving was applied. The chemical nature of the water (Table. 1) shows an abundance of essential components for the growth Ca, Mg, Na, K, which exceeds concentration in the culture medium Zarrouk (1966) [31] and limited concentration reported by Jordan (1999) [32].

Table 1.Characteristics of wastewater and Albian waters. All parameters in mg L^{-1} except pH

Parameter	Wastewater	Albian water
pH	6.80	8.08
Salinity	6620	1890
Ca^{2+}	645	165
Mg^{2+}	601.6	84.5
Na^{+}	2715	262.5
NH_4^{+}	30.26	-
K^{+}	200	45
Cl^{-}	4550	455
SO_4^{2-}	2800	481
PO_4^{-}	8.08	-
HCO_3^{-}	85.40	173.80
NO_3^{-}	5.03	-

2.3 *Evaluation of growth*

The cell growth was measured each 48 hours by absorbance readings (DO) at 625 nm in Spectrophotometer [18]. Cell concentration were measured according to these equations: $Cb=5.32\times DO_{625}$ (WWM) and $Cb=3.4\times DO_{625}$ (CWM), the equations were elaborated from the correlation of absorbance readings and lyophilized biomass for each culture.

Biomass productivity (g L^{-1} d^{-1}) was calculated through the variation in biomass concentration (g L^{-1}) within a cultivation time (*t*) according to the following equation [33]:

$$P=(x_1-x_0)/(t_1-t_0)$$

Where x_1 and x_0 were the biomass concentrations (g L^{-1}) on day t_1 and t_0, respectively.
The pH of the growth medium was measured at each 72 hours. Using a portable pH meter (WTW 315 series) with a combination electrode. The salinity and temperature were measured using a portable electrical conductivity meter (WTW 315 series) with a combination electrode.

2.4 Determination of nitrogen removal as protein content

In this study the efficiency of Spirulina (strain of Tamanrasset) on the removal of N was evaluated by the protein content. The protein content was estimated by the kjeldhal method [34] using a conversion factor of 6.25 [35,36]

3. Results and discussion

3.1 Growth of algal cells

Our experiments were carried out in a spring season under shelter condition (between Mars and April 2013), which show a difference in air temperature of 20 °C. The temperature of culture ranged between 21.03-42.17 °C and averaged 30.23±6.23.

The growth kinetics is presented in terms of biomass concentration, salinity and pH variation. The evolution of the average salinity and pH value are shown in Figure 1, the salinity of the both culture mediums were increased regularly due to the evaporation. The pH values increase in all cultures ranged between 9.48-11.85 and 8.94-12.17

for (CWM) and (WWM) respectively. These pH values were found harmful to culture because they are superior to 10.30 [37, 38,39], but some authors have reported that Spirulina, even at very high pH values outside of the membrane, it can develop, as it is able to maintain a pH gradient appreciable across cytoplasmic membrane [40. 41]. However, an external pH of greater than 10.5 seemed to cause the slow collapse of internal pH homeostasis, especially with regard to sodium depletion, as suggested by Schlesinger et al (1996) [42]. This increase in pH can be correlated to the carbon source consumption [43,44,45]. The bicarbonate ions are assimilated by *A. platensis* and subsequently converted into carbon dioxide and carbonate; an increase in the pH of the system is generated due to the shift of the bicarbonate-carbonate equilibrium, which is required for rapid cell growth [44,46,47,48]. Moreover, the tendency of pH to rise is related to photosynthetic activity, which means that pH becomes higher where photosynthetic activity is higher [49].

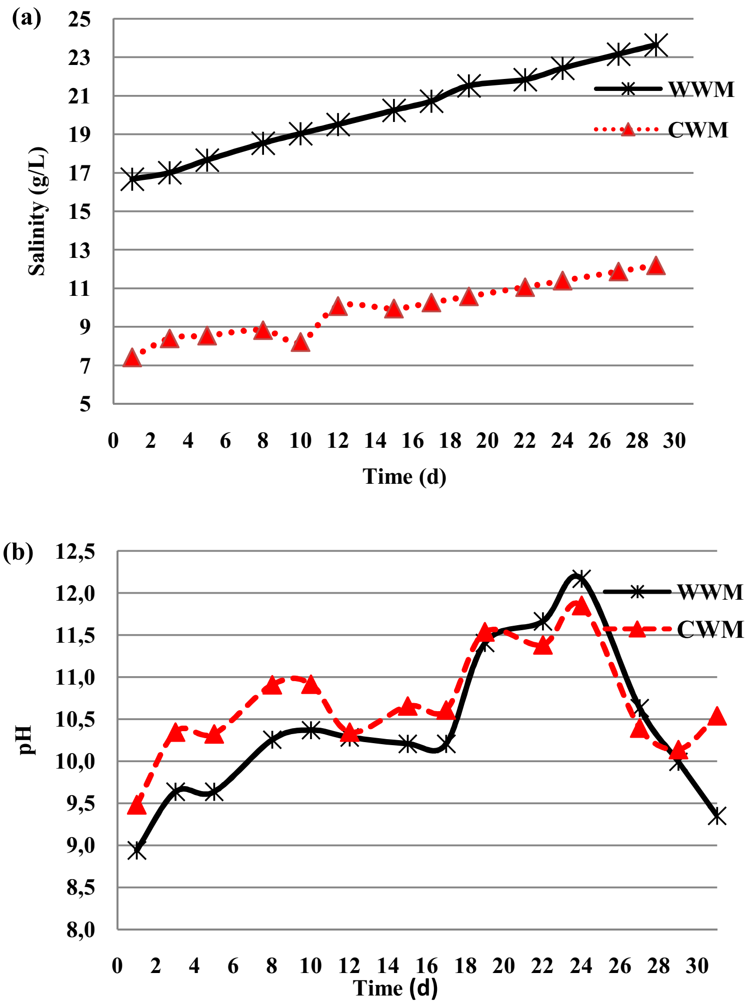

Fig .1. Evolutions of (a) salinity (g L^{-1}) ; (b) pH for both medium

As similar as other microbian growth process, most microalgal growth has four different growth stages: lag phase, exponential or log phase, stationary phase and death phase [50]. The wastewater was characterized as having a high salinity. The algal cells without acclimation in wastewater with a dilution ratio of 0.25 could barely survive. They died and their color turned slightly brown as the ratio increased to 0.45 [10]. However, in our study, the range of salinity was not high enough to stop growth. The WWM medium showed a lag period then a new steady state is established, this due to the shift in salinity value between the inoculums to the WWM medium from 7 to 16.67 g L^{-1}, which results in an immediate cessation of growth and an inhibition after the exposure at the high salt concentration. It has been suggested that when the microalgae were exposed to high salinity wastewater, a high demand for energy is needed to conserve the homoeostasis of the intracellular milieu and maintain metabolic activity [51,52,53]. Time lag is exponentially correlated to the degree of stress imposed on the cells. However, some researchers reported that *S.platensis* had no lag phase [54,55]. As reported Vonshak (1997) [51], the response of Spirulina to salinity with regard to degree of growth inhibition, adaptability to salt levels and the rate of adaptation varies widely, depending on the strain used in the study.

At interval of 7 days, a harvesting (H1, H2, H3, H4) was applied, after each harvesting, a decline growth was observed (Fig. 2) then a recovery phase, this probably explained by the consumption of the nutriments by Spirulina for the synthesis of new biomass which depleted at the end of the experiment and demonstrated by the decline in growth.

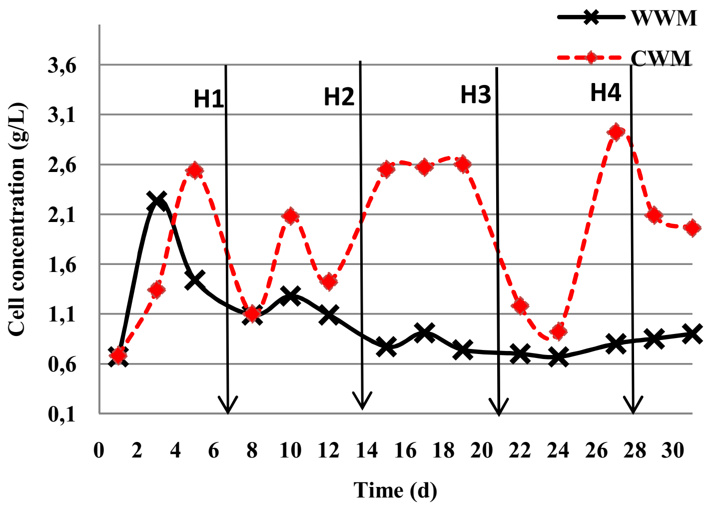

Fig. 2. Evolution of cell concentration (g L^{-1})

As shown in figure 2, the cell concentration evolution in both mediums displays a different profile. The WWM medium characterized by the highest value of maximum biomass productivity with the average of 0.66±0.09 g L^{-1} d^{-1}. This productivity was quite high compared with values found in the literature. It varied from 0.15 to 0.17 g L^{-1} d^{-1} for cultures in Erlenmeyer flasks [56,57], and 0.06 g L^{-1} d^{-1} in outdoor bioreactor [58], and 0.23 g $L^{-1}d^{-1}$ in labscale photobioreactor [59], and from 0.01 to 0.05 g L^{-1} d^{-1} in open ponds [55,60,61]. The WWM medium were the most suitable for steady growth and produced the better biomass productivity and cell concentration at the end of cultures with 0.06±0.01 and 0.96±0.51 g L^{-1} d^{-1} respectively, this medium show exponential growth up to 31th day. While CWM medium grew faster during the first 4 days of culturing, with the cell concentration increasing from the initial 0.67±0.08 to 2.23±0.48 g L^{-1} with an increasing in day 3, however, by day 8, the cell concentration had diverged (1.09±0.25 g L^{-1}), especially when compared this with WWM medium, which could describe the potentiality of the mediums to lead to increased growth and to kept the exponential or stationary phase.

3.2 Nitrogen removal as protein content

An optimization of the culture of *S.platensis* in tertiary effluents improved the conversion of environmental nutriments into microbial protein and resulted in a microalgal biomass with a specific chemical composition. It is generally recognized that the protein content of microalgae depends on the amount of nitrogen available in the culture medium; an increase in nitrogen concentration in the medium is generally parallel to an increase in cellular protein content [62,63,64,65]. Samorı et al (2013) [33] confirmed the correlation between the nitrogen concentration in the medium and the nitrogen concentration incorporated in the biomass as protein by the microalgae.

Protein content was also influenced by the salinity of the medium as Ravelonandro et al (2011) reported [45]. The protein content decreased from 50 ± 2% for a salinity of 13 g L^{-1} to 38 ± 1% for a salinity of 35 g L^{-1}, whereas some found an augmentation of protein contents with salinity [66]. According to some authors, the highest crude protein content in *Spirulina sp.* was achieved at 30 °C combined with a pH value of 9 [39,54,67]. In our experiment, the protein content represents a portion of N removal. As Voltolina et al (2005) reported [26], the addition of 10% to protein content could use to calculate the cell-content organic nitrogen because of the presence of non-protein nitrogen reserves and structures. The condition of the harvested samples tested (H^1) and protein contents were given in Table 2 below:

Table. 2. Condition of the harvested samples tested (H^1)

(H_1)	pH	T (°C)	Salinity (g L^{-1})	Cell concentration g L^{-1}	Protein %
CWM	10.91	26.43	8.83	1.09±0.25	33.54±3.33
WWM	10.26	26.43	19.68	1.10±0.39	28.16±1.73

When comparing the contents of proteins found in both medium (Table. 2) with other classic foods given by Henrikson (1994) such as, chicken meat (24%), beef and fish (22%) [68], we find that they present contents among them. This could allow to the biomass produced in WWM medium to be incorporated into animal feeding. As several factors such as temperature, salinity, gas solubility and availability of nutrients in the culture medium influence not only productivity but also the chemical composition of Spirulina [69,70,71]. Furthermore another important factor is the ratio of each ion to Spirulina, the K / Na ratio, C/N ratio and N/P. Changes in the sources and concentration of nitrogen were accompanied by variation in phosphorus concentration in order to maintain a constant N/P ratio, because the alteration of the N/P ratio can cause more drastic effects on the microalgal culture than the variation in nitrogen concentration [72]. For several algae, the high proportion of proteins is reflected by a low C/N values especially if compared with those of terrestrial plants; C/N ratio can be probably attributed [33]. Also pointed out by Voltolina et al (2005), that high cell concentrations result in low protein contents.

A potential limitation of any assimilative nutrient removal processes in our WWM medium despite of the enhancement of the effluents could be caused by an imbalance of N/P compared to the N/P ratio in the cell tissues as Rhee (1978) [72] and Samorı G et al (2013) reported [33].

4. Conclusion

The comparison between the conventional water medium CWM and wastewater medium WWM might lead to an overvaluation that wastewater maintained algae in exponential growth. This suggests that *S.platensis* can acclimate with new environments and the growth in the wastewater demonstrated that this algal strain is adaptable. It appears to be suitable candidates for cultivation in wastewaters at the studied environmental conditions. In future studies, we hope to further assess the suitability, preferability and possibility to integrate microalgal culture for treatment saline water table by Spirulina.

References

[1] Rouvillois Brigol M. Le pays de Ouargla (Sahara algérien). Variations et organisation d'un espace rural en milieu désertique. Département Géographie Université Paris-Sorbonne, Paris 1975 ;389 p.

[2] Hamdi Aïssa B, Halitim A, Bensaad A. Halilat MT, Daddi-Bouhoun M. Gestion de l'eau pour une agriculture durable au Sahara algérien : INRS-eau université du Québec & université de Poitier eds, Colloque international ESRA' 2000 : Eaux Souterraines en Région Agricole, Poitier, France 2000 : 63-66.

[3] Idder T. Le problème des excédents hydriques à Ouargla : situation actuelle et perspectives d'amélioration. Sécheresse 2007 ;18: 161-7.

[4]Saker ML, Daddi Bouhoun M. Ressources naturelles sahariennes, gestion et impact sur le développement. Annales de la faculté dessciences et science de l'ingénieur 2007;1 :58-62

[5] Oswald WJ, Gotaas HB. Photosynthesis in sewage treatment. Trans Am Soc Civ Eng 1957;122: 73–105.

[6] Olguín,E J. Phycoremediation: key issues for cost-effective nutrient removal processes. Biotechnol Adv 2003; 22:81–91.

[7] Rawat I, Ranjith Kumar R, Mutanda T, Bux F. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production.. Appl Energ 2010;88:3411–3424.

[8]Hu HY, Li X, Yu Y, Wu Y-H, Sagehashi M, Sakoda A. Domestic wastewater reclamation coupled with biofuel/biomass production based on microalgae: a novel wastewater treatment process in the future. J Water Environ Technol 2011;9:199–207.

[9] Abdel Raouf N, Al Homaidan AA, Ibraheem IBM. Microalgae and wastewater treatment. Saudi Journal of Biological Sciences 2012;19:257-

75.
[10] Sun X, Wang C, Li Z, Wang W, Tong Y, Wei J. Microalgal cultivation in wastewater from the fermentation effluent in Riboflavin (B2) manufacturing for biodiesel production. Bioresource Technology 2013;143:499–504.
[11] Chen G, Zhao L, Qi Y. Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: A critical review. Applied Energy 2015; 137:282–91.
[12] Hernandez JP, De Bashan LE, Bashan Y. Starvation enhances phosphorus removal from wastewater by the microalga *Chlorella sp*. coimmobilized with Azospirillum brasilense. Enzyme and Microbial Technology 2006;38:190–98.
[13] Hameed MSA. Effect of algal density in bead, bead size and bead concentrations on wastewater nutrient removal. African Journal of Biotechnology 2007;6:1185–1191.
[14] Henrikson R. Earth Food Spirulina: Ronore Enterprises USA; 1989.
[15] Pulz O, Gross W. Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 2004;. 65: 635–648.
[16] Spolaore, P, Joannis Cassan C, Duran E, Isambert A. Commercial applications of microalgae: a review. J Biosci Bioeng 2006;101:87–96.
[17] Fox RD. Spirulina production and potential. Edisud : Aix-en-Provence ; 1996.
[18] Doumandji A, Boutekrabt L, Saidi NA, Doumandji S, Hamerouch D, Houari S. Etude de l'impact de l'incorporation de la spiruline sur les propriétés nutritionnelles, technologiques et organoleptiques. Nature & Technologie 2012 ;6: 40-50
[19] Sili C, Torzillo G, Vonshak A. *Arthrospira* (Spirulina). In: Whitton BA, editors.Ecology of Cyanobacteria II: Their Diversity in Space and Time: Springer; 2012.p. 677-705
[20] Sood A , Renuka N , Prasanna R, Ahluwalia AS. In: Ansari AA, Gill SS, Gill R, Lanza GR, Newman L, editors . Cyanobacteria as Potential Options for Wastewater TreatmentA.. Phytoremediation: Management of Environmental Contaminants. DOI 10.1007/978-3-319-10969-5_8, © Springer International Publishing Switzerland; 2015.p. 83-93
[21] Becker EW. Micro-algae as a source of protein. Biotechnology Advance 2007;25:207-10
[22] Canizares RO, Dominguez AR. Growth of *Spirulina maxima* on Swine. Bioresource Technology 1993; 45 :73-5
[23] Åkerström AM, Mortensen LM, Rusten B, Gislerød HR. Biomass production and nutrient removal by *Chlorella sp*. as affected by sludge liquor concentration. Journal of Environmental Management 2014;144:118- 124 [24] Chiu SY, Ka CY, Chen TY, Chang YB, Kuo CM , Lin CS. Cultivation of microalgal Chlorella for biomass and lipid production using wastewater as nutrient resource. Bioresource Technology. 2014; biortech.2014.11.080.
[25] Voltolina D, Cordero B, Nieves M, Soto LP. Growth of Scenedesmus in artificial wastewater. Biores Technol 1998;68:265–268.
[26] Voltolina D, Gomez Villa H, Correa G. Nitrogen removal and recycling by *Scenedesmus obliquus* in semicontinuous cultures using artificial wastewater and a simulated light and temperature cycle. Bioresource Technology 2005; 96:359–362.
[27] Phang SM, Miah MS, Yeoh BG, Hashim MA. Spirulina cultivation in digested sago starch factory wastewater Journal of Applied Phycology 2000;12: 395–400.
[28] Lodi A, Binaghi L, Solisio C, Converti A, Del Borghi M. Nitrate and phosphate removal by *Spirulina platensis*. J Ind Microbiol Biotechnol 2003;30: 656–660.
[29] Markou G, Chatzipavlidis I, Georgakakis D. Cultivation of *Arthrospira* (Spirulina) *platensis* in olive-oil mill wastewater treated with sodium hypochlorite. Bioresource Technology 2012;112:234–241.
[30] Hiri A. Petite nouvelle de la spiruline. Antenna Tech. France ;2011
[31] Zarrouk C. Contribution à l´étude d´une cyanophycée influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch. et Gardner) Geitler. Thèse doctorat. Université de Paris :06/12/1966.
[32] Jordan J P. Cultivez votre spiruline : manuel de culture artisanal .Publication Antenna Technologies.Geneve:1999.
[33] Samorı G, Samorı C, Guerrin F, Pistocchi R. Growth and nitrogen removal capacity of *Desmodesmus communis* and of a natural microalgae consortium in a batch culture system in view of urban wastewater treatment: Part I. Wastewater research : 2013 ; 31 :7 9 1-8 0 1.
[34] APHA. Standard methods for the examination of water and wastewater. American Public health association, Washington DC. 2005.
[35] Göksan T, Zekeriyaoğlu A, Ak I. The growth of *Spirulina platensis* in different culture systems under greenhouse condition. Turk J Biol : 2007 ;31 :47-52.
[36] Coca M, Barrocal VM, Lucas S, González Benito G, García Cubero MT. Protein production in *Spirulina Platensis* biomass using beet vinasse-supplemented culture media. Food and bioproducts processing 2014; FBP-492.
[37] Richmond A, Vonshak A, Arad SM. Environmental limitations in outdoor production of algal biomass. In: Shelef G, Soeder CJ, editors.Algae. Biomass, Amsterdam,: Elsevier/North Holland Biomedical Press; 1980. p.65-72.
[38] Binaghi L, Del Borghi A, Lodi A, Converti A, Del Borghi M. Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis*. Process Biochem 2003;38:1341–6.
[39] Ogbonda KH, Aminigo RE, Abu GO. Influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina sp*.. Bioresour Technol 2007;98:2207–11.
[40] Krulwich TA; Guffanti A A. Alkalophilic bacteria. Annual Review of Microbilogy 1989; 43: 435-463.
41] Belkin S, Boussiba S. Resistance of *Spirulina platensis* to ammonia at high pH values. Plant Cell Physiol. 1991; 32:953-58. 8 Author name / Energy Procedia 00 (2015) 000–000 [42] Schlesinger P, Belkin S, Boussiba S. Sodium deprivation under alkaline conditions causes rapid death of the filamentous cyanobacterium *Spirulina platensis*. J Phycol 1996;32:608- 13.
[43] Fox RD. Spiruline Technique, pratique et promesse.Aix-en-Provence :EDISUD ;1996
[44] Volkmann H, Imainovsky E, Badial F J, Barcelos Oliveira J L, Sebastião, Sant'anna E. Influence of desalinatore wastewaster for thecultivation of Arthrospira palatensis. Grasas y aceites 2007;58: 396-401.
[45] Ravelonandro PH, Ratianarivo DH, Joannis-Cassan C, Isambert A, Raherimandimby M. Improvement of the growth of *Arthrospira* (Spirulina) *platensis* from Toliara (Madagascar): Effect of agitation, salinity and CO 2 addition. Food and Bioproducts Processing 2011:89: 209-216
[46] Miller A G, Colman G. Evidence for HCO3 transport by the blue green alga (cyanobacterium) *Coccochloris peniocystis*. Plant Physiol 1980;65: 397-402.
[47] Badger MR. G.D. P. CO2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. J Exp Bot 2003;54:609–22.

[48] Kim CJ, Jung YH, Oh HM. Factors indicating culture status during cultivation of *Spirulina* (Arthrospira) *platensis*. J Microbiol 2007;45:122–27.
[49] Andrade MR, Costa JAV. Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture 2007;264:130–4.
[50] Zhao B, Su Y. Process effect of microalgal-carbon dioxide fixation and biomass production. Renewable and Sustainable Energy Reviews 2014; 31: 121-132.
[51] Vonshak A. Arthrospira (Spirulina): systematics and ecophysiology biochemistry. In: Vonshak A, editor. *Spirulina platensis* (Arthrospira): physiology, cell biology and biotechnology. London: Taylor & Francis; 1997 p: 43-65.
[52] Rafiqul IM, Hassan A, Sulebele G, Orosco CA, Roustaian,P, Jalal KCA. Salt stress culture of blue-green algae *Spirulina fusiformi*. Pakistan Journal of Biological Sciences 2003;6:648–50.
[53] Eggert A, Nitschke U, West JA, Michalik D, Karsten U. Acclimation of the intertidal red alga *Bangiopsis subsimplex* (Stylonematophyceae) to salinity changes. Journal of Experimental Marine Biology and Ecology 2007;343:176–86.
[54] Colla LM, Reinehr CO, Reichert C, Costa JAV. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioresour Technol 2007; 98:1489–93
[55] Radmann EM, Reinehr CO, Costa JAV. Optimization of the repeated batc cultivation of microalga *Spirulina platensis* in open raceway ponds. Aquaculture 2007;265:118–26.
[56] Pelizer LH, Danesi ED, Rangel CO, Sansano CEN, Carvalho JCM, Sata S, Moraes IO. Infuence of inoculums age and concentration in *Spirulina platensis* cultivation. J Food Eng 2003;56:371-75.
[57] Wang CY, Fu CC, Liu YC. Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. Biochem Eng J 2007; 37:21–25.
[58] Lanlan Z, Lin C, Junfeng W, Yu C, Xin G, Zhaohui Z, Tianzhong L. Attached cultivation for improving the biomass productivity of *Spirulina platensis*. Bioresource Technology 2015; 181:136–42.
[59] Ruiz Martinez A, Martin Garcia N, Romero I, Seco A, Ferrer J. Microalgae cultivation in wastewater: Nutrient removal from anaerobic membrane bioreactor effluent. Bioresource Technology 2012;126: 247–53.
[60] Olguìn EJ, Galicia S, Camacho R, Mercado G, Pérez TJ. Production of *Spirulina s*p. in sea water supplemented with anaerobic effluents in outdoor raceways under temperate climatic conditions. Appl Microbiol Biotechnol 1997;48:242–47.
[61] Ravelo V. Bio-écologie, valorisation du gisement naturel de Spiruline de Belalanda (Toliara, sud-ouest de Madagascar) et technologie de la culture. PhD Thesis. University of Toliara, Madagascar:2001.
[62] Venkataraman LV., Nigan BP. Mass culturing of freshwater algae for utilization as protein source. Phykos 1979; 18: 83-95.
[63] Boussibs S, Richmond AE. Phycocyanin as a storage protein in the blue-green alga *Spirulina platensis*. Arch Microbia 1980; 125: 143-147.
[64] Göksan T, Zekeriyaoğlu A, Ak I. The growth of *Spirulina platensis* in different culture systems under greenhouse condition. Turk J Biol : 2007 ;31 :47-52.
[65] Sassano CEN, Gioielli LA, Ferreira LS, Rodrigues MS, Sato S, Converti A, Carvalho JCM. Evaluation of the composition of continuously cultivated *Arthrospira* (Spirulina) *platensis* using ammonium chloride as nitrogen source. Biomass Bioenergy 2010;34:1732–38.
[66] Rosales N, Ortega J, Mora R, Morales E. Influence of salinity on the growth and biochemical composition of the cyanobacterium *Synechococcus sp*. Cienc Mar 2005;31:349–55.
[67] De Oliveira MACL, Monteiro MPC, Robbs PG, Leite SGF. Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. Aquacult Int 1999;7:261–75.
[68] Henrikson R. Microalga Spirulina, superalimento del futuro, Ronore Enterprises.2ª ed. Ediciones Urano. Barcelona: España:1994
[69] Olguin EJ. The cleaner production strategy applied to animal production. In: Olguin EJ, Sanchez G, Hernandez E, editors. Environment biotech. and cleane processes.London; Taylor & Francis:2000.p. 227-241.
[70] Tang DS, Shi SY, Li DH, Hu C, Liu Y. Physiological and biochemical responses of *Scy-tonema javanicum* (cyanobacterium) to salt stress. Journal of Arid Environments 2007;71:312–20.
[71] Markou G, Georgakakis D. Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review. Applied Energy 2011;88:3389–3401.
[72]Wynne D, Rhee GY. Effects of light intensity and quality on relative N and P requirement (the optimum N:P ratio) of marine planktonic algae. J Plankton Res 1986;8: 91-103.