

Microalgae as a Valuable Additive in Aquaculture

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Abstract-In the last few decades, aquaculture is a fast growing sector. Increasing requirements for protein and high cost of fishmeal, has led to new alternatives as animal and plant source of additives in aquaculture. In aquaculture industry, fishmeal is the first preferred protein source of nutrition. Decreasing fishmeal supply, contributing to the variable cost, threaten the sustainability and growth of the aquaculture industry. Microalgal biomass has gained importance and its use is extending in several areas such as bioremediation, aquaculture, nutraceutical research, renewable energy and biomolecule sources. In recent decades, microalgae are used as alternative protein ingredient replacing fishmeal. Microalgae have a good profile of nutrient composition and have gained popularity due to their appropriate size, high growth rate, resistance to diseases and antioxidant activity. Microalgae used frequently in feeding are *Scenedesmus*, *Chlorella*, *Pavlova*, *Tetraselmis* and *Chaetoceros* sp. In the present study, the importance of microalgae for aquaculture was compiled to take more attention due to the species that are valuable additive in feeding as an alternative nutrition source.

Keywords: Aquaculture, Microalgae, Nutrition, Fishmeal.

I. INTRODUCTION

Microalgae are at the first step of the aquatic food chain and play a vital role that are used in rearing of aquatic organisms like shrimps, fishes and mollusks [1]. Microalgae are required diversely in aquaculture for applications in directly or indirectly consumption as prepared feed. The importance of microalgae as a feed supplement in aquaculture is increasing due to their nutritional value, appropriate size, antioxidant activity, high growth rate and resistance to diseases. The nutritional value of these species is according to their size, digestibility, shape and biochemical composition [2].

Microalgae are either prokaryotic or eukaryotic microscopic organisms typically found in freshwater and marine systems growing through photosynthesis. The cell structure of microalgae is simple and their growth requires light, carbon dioxide, water, and nutrients. Photosynthetically, microalgae can convert those necessities into energy and use it in cell development [3]. They are unicellular species which exist individually, in chains or groups. Their sizes can range from a few micrometers (μm) to a few hundreds of micrometers. Unlike higher plants, microalgae do not have roots, stems or leaves. Microalgae, are important for life on earth due to the capable of performing photosynthesis. They produce approximately half of the atmospheric oxygen and use simultaneously the greenhouse gas carbon dioxide to grow photoautotrophically.

Microalgae species adapted for growing conditions in all types of waters (salty, brackish, fresh) and temperatures (ranging from polar to tropical and even extremely hot conditions). They are also adapted to diverse light intensities (surface waters, symbiosis inside animals and deep waters), and are able to survive at pH ranges from 0 to over 11.

Microalgae have a great importance to use for food, production of bioactive compounds, as biofilters to remove pollutants and nutrients from water, in pharmaceutical and cosmetic industry [4]. Also they are a good source for biofuel production due to their high oil content and biomass production [5].

Microalgae can be used to produce a wide range of metabolites such as proteins, lipids, carbohydrates, carotenoids or vitamins. The first use of microalgae by humans dates back 2000 years to the Chinese, who used *Nostoc* to survive during famine. Microalgal technology began to develop in the middle of the last century. In recent years, there have been numerous commercial applications of microalgae such as to enhance the nutritional value of food and animal feed due to their chemical composition [6].

Microalgae have three fundamental attributes that can be converted into technical and commercial advantages. They are genetically diverse group of organisms with a wide range of physiological and biochemical characteristics so they naturally produce many different fats, sugars, bioactive compounds. They comprise a large, unexplored group of organisms, so that they provide a virtually untapped source of products. In recent years, microalgae apart from being used as single-cell proteins, they are projected as living-cell factories for the production of bio-fuels and various beneficial biochemicals used in food, aquaculture, poultry and pharmaceutical industries due to presence of different beneficial bioactive compounds [7].

II. NUTRITIONAL VALUE OF MICROALGAE

The chemical composition of microalgae give us the basic information about the nutritive potential of algal biomass. The nutritional value of a microalgae is related to its ability to supply essential macro and micronutrients to the diets. Microalgae contain numerous bioactive compounds that can be utilized for commercial use. They have been used successfully to produce high concentration of valuable compounds such as carbohydrates, lipids, proteins and other pigments [8].

Factors contribute to the nutritional value of microalgae species due to their size and shape, digestibility as related to cell wall structure, biochemical composition and requirements of the diet [9].

Studies indicated that microalgae contain 30-40 % protein, 10-20 % lipid and 5-15 % carbohydrates [10]. In cultured phase of microalgae the proximate composition of the species may significantly change [11]. Microalgae provide many nutrients including PUFAs (EPA, arachidonic acid and DHA) which are essential for marine animals diets and for growth of many larvae [12; 7].

Carbohydrates play an important role in digestibility of the total algal biomass [13]. Carbohydrates of algae are found in the form of starch, cellulose, sugars and other polysaccharides. Twelve species of microalgae of Bacillariophyceae and Chlorophyceae contain carbohydrate [14]. Lewin (1956) reported that *Chlamydomonas* spp. for extracellular

polysaccharide production [15]. The most useful species is *C. mexicana* yields up to 25 % of its total production of as polysaccharides [16]. Ramus (1972) indicated the production of polysaccharides from green and blue-green algae and unicellular red algae *Porphyridium cruentum* and *P. aeruginosum*[17]. Some microalgae commonly used in aquaculture are *Pyramimonas virginica*, *Pseudoisochrysis paradoxa*, *C. vulgaris*, *P. lutheri* and *Isochrysis galbana* and their major glucose constituent are mannose, ribose/xylose, rhamnose and fucose accounted for 28–86 % of the total carbohydrates[18].

Fatty acid compositions of microalgae were first studied in the 1940s [19]. More than 100 microalgae species have been analyzed for fatty acid composition. These fatty acids are essential components of the diets and are becoming important feed additives in aquaculture [1]. Algal lipids are found in the form of glycerol, sugars or bases, esterified to fatty acids may be saturated or unsaturated. Triglycerides are the common form of lipids of 80 % in Cyanobacteria [20].

The major cellular lipids of blue green algae include monogalactosyldiglyceride, digalactosyldiglyceride and sulfoquinovosyldiglyceride [21]. Kenyon (1972) reported the fatty acid profile of unicellular blue green algae belonging to the genera *Synechococcus*, *Aphanocapsa*, *Gloeocapsa*, *Microcystis* and *Chlorogloea* [22]. Volkman et al. (1989) reported PUFA (EPA) in four genera of diatoms. *Chaetoceros calcitrans*, *Chaetoceros gracilis*, *Skeletonema costatum* and *Thalassiosira pseudonana* (4–11 % of the total fatty acids), the cryptomonad *Chroomonas salina* (11, 12 %) and the prymnesiophyte *P. lutheri* (20 %) [23]. Other major algal lipids are lecithin, phosphatidyle glycerol and inositol. Lipids are highly significant at various growth stages of many aquaculture animals including marine fish larvae, bivalves, mollusks etc. Choi et al. (1987) reported complete lipid profile of *Scenedesmus obliquus* with 12 % total lipid and neutral lipid, glycolipid and phospholipid fractions at 7, 2.45 and 1.48 % level on dry weight [24].

The quality of proteins can vary depending on digestibility and the availability of essential amino acids[25]. Animal sources protein are usually considered as complete proteins, due to their rich source of essential amino acids which the human body can not to biosynthesize. Alternatively, plant proteins are often considered an incomplete protein source as they generally lack some essential amino acids [26]. Seaweed and microalgae are considered as a source of protein. Some species of seaweed and microalgae are known to contain protein levels similar to those of common protein sources, like meat, egg, soybean, and milk[27]. High arginine content was reported in *Tetraselmis suecica*, *T. chuii* and *Pavlova lutheri* as superior in nutritional quality [28]. *Spirulina platensis* has nearly similar amino acid profile as egg, better depending on the culture condition [29].

Microalgae are a good source of variable vitamins. A number of vitamins are present in higher levels in algae than other common rich conventional sources [30]. The vitamin profile of several algae are investigated over the years. For example, *Spirulina platensis*, *S. maxima*, *Scenedesmus obliquus*(Becker 1984; Jaleel and Soeder 1978), *Aphanizomenon flos aquae*) etc. [29, 31, 32, 33]. Brown et al. (1999) investigated the vitamin content of some Australian microalgae like *Nannochloropsis*, *Pavlova*, *Strichococcus*, *Tetraselmis*, *Chaetoceros*, *Thalassiosira* and *Isochrysis* sp. [34]. Concentrations of vitamins typically show a difference between species [35]. Brown (2002) suggested that mixed-algal

diet should provide adequate concentrations of the vitamins for aquaculture food chain [36].

III. THE USE OF MICROALGAE IN AQUACULTURE

It has become essential to search for alternative sources of feed additives due to the increasing cost of animal feeds. Microalgae are the natural base of the aquatic food chain. This has led to their widespread incorporation as an important food source and feed additive for many aquatic animals, including molluscs, shrimp, and rotifers[37]. Microalgae also play an important role in aquaculture, other than as a food source for zooplanktons to stabilise pH, reduce bacterial growth, and improve the quality of rearing medium [38].

The use of algae as an additive in aquaculture has received attention due to the positive effects on weight gain, increased triglyceride, digestibility and protein deposition in muscle, improved resistance to disease, decreased nitrogen output to the environment, increased physiological activity and starvation tolerance [39]. Microalgae live in complex habitats with different conditions of salinity, temperature, nutrients, UV-Vis irradiation. Therefore, they can adapt rapidly to the extreme environmental conditions to survive with producing variety of secondary metabolites which cannot be found in other organisms. Since 1980–1990s, microalgae species have been used in aquaculture, mainly for the production of larvae, juvenile shell and finfish and for raising the zooplanktons required for feeding [40]. Microalgae species are generally used for growth stages of molluscs for the juvenile stages of abalone, crustaceans, fish and zooplanktons in food chain [36].

The nutritional value of microalgae can vary significantly and this may also change under culture conditions. Combination of different species which include *C. calcitrans*, *C. muelleri*, *P. lutheri*, *Isochrysis* sp., *T. suecica*, *S. costatum* and *Thalassiosira pseudonana* provides better balanced nutrition and improves growth better than a diet composed of one algae [41].

Protein and vitamin content is the major factor determining the nutritional value of microalgae, also polyunsaturated fatty acid composition. Possibility of replacement the fishmeal with algae has been investigated by several workers [42, 43, 44]. El-Hindawy et al. (2006) especially in tropical areas that are found in abundant amounts [44]. The high protein content of algae are beneficial for animal feed, including aquaculture, farm animals, and pets. 30% of global algae production is estimated to be used for animal feed, with 50% of *Spirulina* sp. used as feed supplement due to its excellent nutritional profile[45].

Microalgae are also used a natural source of pigments for the culture of fishes and prawns. The species for this usage are *Dunaliella salina*, *Haematococcus pluvialis* and *Spirulina* sp. [46]. *Chlorella* sp. and *Spirulina* sp. are mostly used in feeds where colouration and healthy appearance is the main criteria [47]. Some valuable products extracted from microalgae are astaxanthin and lutein produce from *Haematococcus pluvialis* and *Tetraselmis* sp., phycocyanin from *Spirulina platensis*, polyunsaturated fatty acids from *Chlorella* sp. and *Schizochytrium* sp., biotin and vitamin E from *Euglena gracilis* [48]. Lutein can be converted to the vitamin A to improve the nutritional value of Artemia. The pigment lutein is common in green microalgae (generally *Tetraselmis* sp.). Beta-carotene used as orange dye and supplement of vitamin C is a photosynthetic pigment from *Dunaliella salina*[49].

The history of the commercial use of algae about 75 years with application for wastewater treatment and mass production of different strains such as *Chlorella* and *Dunaliella* [50]. Bio-treatment with microalgae is particularly attractive because of their photosynthetic capabilities, converting solar energy into useful biomasses and incorporating nutrients such as nitrogen and phosphorus [51]. The using of aquaculture systems as engineered systems in wastewater treatment and recycling has increased enormously over the past few years, they are designed to achieve specific wastewater treatment and can simultaneously solve the environmental and sanitary problems and may also be economically efficient. Wastewater from fish farms are enriched with solid particles and dissolved nutrients, mainly in the form of inorganic nitrogen and phosphorus. The use of live microalgae to remove excess dissolved nutrients from aquaculture effluents is an efficient and effective wastewater treatment method [52]. Algae can be used in wastewater treatment for a range of purposes, some of which are used for the removal of coliform bacteria, reduction of both chemical and biochemical oxygen demand, removal of N and/or P, and also for the removal of heavy metals [50].

CONCLUSION

The microalgae are cultivated and use for feed, production of bioactive compounds, as biofilters to remove nutrients and other pollutants from wastewaters, in cosmetic and pharmaceutical industry and in aquaculture. Also microalgae are potentially good sources for biofuel production owing to their high oil content and rapid biomass production. The main application of microalgae in aquaculture is connected with their usage for animal feed. The aquatic habitat and the appropriate size of microalgae species are consumed directly by fishes or indirectly zooplanktons. Algal biomass would play an important role in enhancement of growth performances of fishes, their resistance to diseases and the skin color.

In conclusion, algae have been reported as a valuable source of nutrition. More research is needed to investigate the bioavailability of algal bioactive compounds.

References

- [1] M.A. Borowitzka, 'Microalgae as sources of pharmaceuticals and other biologically active compounds', *Journal of Applied Phycology*, 1998, vol. 4, pp. 267-279.
- [2] M.R. Brown, S.W. Jeffrey, J.K. Volkman, and G.A. Dunstan, 'Nutritional properties of microalgae for mariculture', *Aquaculture*, 1997, vol. 151, pp. 315-331.
- [3] A. SitiZullaikah, U. Tjipto, M.Y. Lu, Y. KiOng, J. Hsu, 'Ecofuel conversion technology of inedible lipid feedstocks to renewable fuel', *Advances in Eco-Fuels for a Sustainable Environment*, Woodhead Publishing Series in Energy, 2019, pp. 237-276.
- [4] I. Sirakov, K. Velichkova, S. Stoyanova, and Y. Staykov, 'The importance of microalgae for aquaculture industry', *International Journal of Fisheries and Aquatic Studies*, 2015, vol. 2(4), pp. 81-84.
- [5] P. Sharma, M.B. Khetmalas, and G.D. Tandon, 'Biofuels from green microalgae', *Biotechnology: Prospects and Applications*, Springer, 2013, pp. 95-112.
- [6] I. Priyadarshani and B. Rath, 'Commercial and industrial applications of micro algae - A review', *Algal Biomass Utln.* vol. 3(4), pp. 89-100.
- [7] W. Becker, 'Microalgae in human and animal nutrition', A. Richmond (Ed) *Handbook of Microalgal Culture*, Blackwell, Oxford, 2004, pp. 312-351.
- [8] I. Zatkova, M. Sergejevova, and J. Urban, 'Carotenoid-enriched microalgal biomass as feed supplement for freshwater ornamentals: Albino form of Wels catfish (*Silurus glanis*)', *Aquaculture Nutrition*, 2011, vol. 17(3), pp. 278-286.
- [9] C. Guedes and F.X. Malcata, 'Nutritional value and uses of microalgae in aquaculture', *Aquaculture*, 2011, pp. 59-78.
- [10] S.M. Renaud, L.V. Thinh, and D.L. Parry, 'The gross composition and fatty acid composition of 18 species of tropical Australia microalgae for possible use in mariculture', *Aquaculture*, 1999, vol. 170, pp. 147-159.
- [11] P. J. Harrison, P.A. Thompson, and G.S. Calderwood, 'Effects of nutrient and light limitation on the biochemical composition of phytoplankton', *Journal of Applied Phycology*, 1990, vol. 2, pp. 45-56.
- [12] D.S. Nichols, 'Prokaryotes and the input of polyunsaturated fatty acids to the marine food web,' *FEMS Microbiology Letters*, 2003, vol. 219, pp. 1-7.
- [13] E.E. Percival, and J.R. Turvey, 'Polysaccharides of algae', In *CRC Handbook of Microbiology*, 532nd edition, CRC Press Inc., 1974.
- [14] S.M. Renaud, D.L. Parry, and L.V. Thinh, 'Microalgae for use in tropical aquaculture I: Gross chemical composition and fatty acid composition of twelve species of microalgae from the Northern Territory, Australia', *Journal of Applied Phycology*, 1994, vol. 6, pp. 337-345.
- [15] R.A. Lewin, 'Extracellular polysaccharides of green algae', *Can. Journal of Microbiology*, 1956, vol. 2, pp. 665-672.
- [16] S.S. Roy and R. Pal, 'Microalgae in Aquaculture: A Review with Special References to Nutritional Value and Fish Diets', *Proc Zool Society*, 2015, vol. 68(1), pp. 1-8.
- [17] J.S. Ramus, 'The production of extracellular polysaccharides by the unicellular red alga *Porphyridium eurugineum*', *Journal of Phycology* 1972, vol. 8, pp. 97-111.
- [18] F.E. Chu, J.L. Dupuy, and K.L. Webb, 'Polysaccharide composition of five algal species used as food for larvae of the American oyster, *Crassostrea virginica*', *Aquaculture*, 1982, vol. 29(3-4), pp. 241-252.
- [19] H.W. Millner, 'The fatty acids of *Chlorella*', *Journal of Biochemistry*, 1948, vol. 176, pp. 813-817.
- [20] T.G. Tornabene, G. Holzer, S. Lien, and N. Burris, 'Lipid composition of the nitrogen starved green alga *Neochloris oleoabundans*', *Enzyme and Microbial Technology* 1983, vol. 5(6), pp. 435-440.
- [21] B.W. Nichols, 'Comparative lipid biochemistry of photosynthetic organisms', *Phytochemical phylogeny*, 1970, pp. 105-118, Academic Press New York.
- [22] C.N. Kenyon, 'The fatty acid compositions of unicellular strains of blue green algae', *Journal of Bacteriology*, 1972, vol. 109, pp. 827-832.
- [23] J.K. Volkman, S.W. Jeffrey, P.D. Nichols, G.I. Rodgers, and C.D. Garland, 'Fatty acid and lipid composition of ten species of microalgae used in mariculture', *Journal of Experimental Marine Biology and Ecology*, 1989, vol. 128, pp. 219-240.
- [24] K.J. Choi, V. Nakhost, J. Krukoni, and M. Karel, 'Supercritical fluid extraction and characterization of lipids from algae *Scenedesmus obliquus*', *Food Biotechnology*, 1987, vol. 1(2), pp. 263-281.
- [25] S. Bleakley and M. Hayes, 'Algal Proteins: Extraction, Application, and Challenges Concerning Production', *Foods*, 2017, vol. 6, pp. 1-33.
- [26] V.R. Young, P.L. Pellett, 'Plant proteins in relation to human protein and amino acid nutrition', *Am. J. Clin. Nutrition*, 1994, vol. 59, pp. 1203-1212.
- [27] L. Gouveia, A.P. Batista, I. Sousa, A. Raymundo, and N. Bandarra, 'Microalgae in novel food products', *Food Chemistry Research Development*, Konstantinos Papadopoulos, P.P., Eds., Nova Science Publishers, New York, USA, 2008, pp. 75-112.
- [28] K.L. Webb, and F.E. Chu, 'Phytoplankton as a food source for bivalve larvae', *Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition*, Louisiana State University, Baton Rouge, LA, 1983, pp. 272-291.
- [29] E.W. Becker, and L.V. Venkataraman, 'Production and utilization of the blue green alga *Spirulina* in India', *Biomass*, 1983, vol. 4, pp. 105-125.
- [30] M.R. Brown, and C.A. Farmer, 'Riboflavin content of six species of microalgae used in mariculture', *Journal of Applied Phycology*, 1994, vol. 6, pp. 61-65.
- [31] T.V. Jaya, M.L. Scarino, and M.A. Spaodoni, 'Caratteristiche nutrizionali in vivo di *Spirulina maxima*', *Prospective dell'acquacoltura di Spirulina in Italia*, ed. I.R. Materassi, 1980, vol. 195, Florence: Firenze-Academia dei Georgofili.
- [32] E.W. Becker, 'Microalgae: Biotechnology and microbiology', Cambridge: Cambridge University Press, 1994.

- [33] S.A. Jaleel, and C.J. Soeder, 'Current trends in microalgal cultures as protein source in West Germany', *Indian Food Packer*, 1978, pp. 27-45.
- [34] M.R. Brown, M. Mular, I. Miller, C. Trenerry, and C. Farmer, 'The vitamin content of microalgae used in aquaculture', *Journal of Applied Phycology*, 1999, vol. 11, pp. 247-255.
- [35] C. Segueineau, A. Laschi-Loquerie, J. Moal, and J.F. Samain, 'Vitamin requirements in great scallop larvae', *Aquaculture International*, 1996, vol. 4, pp. 315-324.
- [36] M.R. Brown, 'Nutritional value of microalgae for aquaculture', *Avances en Nutricio'n Acu'cola VI*. eds. L. E Cruz-Sua'rez, D Ricque-Marie, M Tapia-Salazar, M G Gaxiola-Corte's, N Simoes. *Memorias del VI Simposium Internacional de Nutricio'n Acu'cola*. 3 al 6 de Septiembre del Cancun, Quintana Roo, Mexico, 2002.
- [37] M.A. Borowitzka, 'Microalgae for aquaculture: Opportunities and constraints', *Journal of Applied Phycology*, 1997, vol. 9, pp. 393-401.
- [38] G.D. Lio-Po, E.M. Leañó, M.D. Peñaranda, A.U. Villa-Franco, C.D. Sombito, and N.G. Guanzon, 'Anti-luminous vibrio factors associated with the 'green water' grow-out culture of the tiger shrimp *Penaeus monodon*', *Aquaculture*, 2005, vol. 250, pp. 1-7.
- [39] J. Fleurence J, M. Morancais, J. Dumay, P. Decottignies, V. Turpin and M. Munier, 'What are the prospects for using seaweed in human nutrition and for marine animals raised through aquaculture?', *Trends Food Sci Technology*, 2012, vol. 27, pp. 57-61.
- [40] E.O. Duerr, A. Molnar, and V. Sato, 'Cultured microalgae as aquaculture feeds', *Journal of Marine Biotechnology*, 1998, vol. 75, pp. 65-70.
- [41] P.C. Spolaore, E.D. Joannis-Cassan and A. Isambert, 'Commercial application of microalgae', *Journal of Bioscience and Bioengineering*, 2006, vol. 101, pp. 87-96.
- [42] W. Broun, 'Note on the survival of algal resting cells during long-term maintenance in darkness and minimum lake bottom temperature', *Comparison of Anabaena*, 1980, vol. 5, pp. 677-680.
- [43] H.N. Appler, 'Evaluation of *Hydrodictyon reticulatum* as protein source in feeds for *Oreochromis niloticus* and *Tilapia zillii*', *Journal of Fish Biology*, 1985, vol. 27(3), pp. 327-334.
- [44] M.M. El-Hindawy, M.A. Abd-Razic, H.A. Gaber and M.M. Zenhom, 'Effect of various level of dietary algae *Scenedesmus* spp. on physiological performance and digestibility of *Nile tilapia* fingerlings, 1st Scientific Conference of the Egyptian Aquaculture Society, Sinai: Sharm El-Sheikh, 2006, pp. 137-149.
- [45] N.T. De Marsac, 'Phycobiliproteins and phycobilisomes: The early observations', *Photosynth. Res.*, 2003, vol. 76, pp. 193-205.
- [46] H.H. Abd El-Baky, A. Moawd, A.N. El-Behairy, G.S. ElBaroty, 'Chemoprevention of benzo[a]pyrene-induced carcinogen and lipid peroxidation in mice by lipophilic algae extracts (phycotene)', *J Med Science*, 2002, vol. 2, pp. 185-193.
- [47] L. Gouvei and P. Rema, 'Effect of microalgal biomass concentration and temperature on ornamental goldfish (*Carassius auratus*) skin pigmentation', *Aquaculture Nutrition*, 2005, vol. 11(1), pp. 19-23.
- [48] Y. Li, M. Horsman, N. Wu, C.Q. Lan, and N. Dubois-Calero, 'Biofuels from Microalgae', *Biotechnology Progress*, 2008, vol. 24, pp. 815- 820.
- [49] I. Rnnestad I, S. Helland, Q. Lie, 'Feeding artemia to larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) results in lower larval vitamin A content compared with feeding copepods', *Aquaculture*, 1998, vol. 165, pp. 159-164.
- [50] N. Abdel-Raouf, A.A. Al-Homaidan, I.B.M. Ibraheem, 'Microalgae and wastewater treatment', *Saudi Journal of Biological Sciences*, 2012, vol. 19, pp. 257-275.
- [51] S. Sebastian, and K.V.K. Nair, 'Total removal of coliforms and *E. coli* from domestic sewage by high-rate pond mass culture of *Scenedesmus obliquus*', *Environmental Pollution (Series A)*, 1984, vol. 34, pp. 197-206.
- [52] K. Velichkova, I. Sirakov, S. Stoyanova, 'Biomass production and wastewater treatment from aquaculture with *Chlorella vulgaris* under different carbon sources', *Scientific Bulletin. Series F. Biotechnologies*, 2014; vol. 18, pp. 83-88.