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A Review of Moroccan Microalgae and Their Exploitation Fields

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Abstract:
Microalgae are microscopic and ubiquitous organisms that present an enormous biodiversity. They have the ability to perform photosynthesis and produce about half of the atmospheric oxygen. These microorganisms produce also different biologically active metabolites that give them a large number of applications such human and animal nutrition, cosmetics, pharmaceutical, biofuels production, environmental management such as wastewater treatment, bioremediation, capture of CO_2 and in agriculture as atmospheric N_2-fixer, phosphate solubiliser and source of various plant-growth biostimulant. Recently, interest in microalgae research has increased in various fields and the microalgae sector is very dynamic throughout the world. Nevertheless, it is still under-exploited in Morocco, even if the potential remains important due to favorable climatic conditions and to its specific geographical position: The Mediterranean Sea to the north, the Atlantic Ocean to the west. There are five new industries producing only Spirulina and derivatives. This review presents an overview of the main research works that have been conducted on microalgae in Morocco, the biodiversity of isolated species and their different areas of exploitation.

***Key Word**: Microalgae; biodiesel production; biostimulant; wastewater treatment; antibacterial activity, Morocco.*

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I. Introduction

Microalgae are unicellular, microscopic (2–200 µm), photosynthetic organisms that grow in a range of aquatic habitats, including saline and freshwater environments[1,2]. They are mostly eukaryotic although the prokaryotic cyanobacteria (blue-green algae) are frequently classified as algae[3,4]. They are ubiquitous with high adaptivity even under harsh environments such as high temperature or high salinity[5]. It has been estimated that over 800,000 species of microalgae exist. However, only about 50 000 have been described in the literature underscoring their incredible diversity[6]. Classification of microalgae has traditionally been conducted by pigmentation, life cycle, and basic cellular structures, nevertheless, molecular methods such as denaturing gel electrophoresis and next-generation sequencing are emerging techniques in detecting and classifying new species of microalgae[4,7]. They are categorized into four main classes in terms of abundance: diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), blue-green algae i.e., *Spirulina* (*Arthrospira platensis* and *Arthrospira maxima)* and golden algae (*Chrysophyceae*)[1]. In recent decades, there has been a great trend for research and industrial applications of marine compounds and biotechnology[8,9]. Among the large spectrum of marine organisms, microalgae represent a promising source for several bioactive compounds that give them a large number of applications in various fields such as human or animal nutrition, pharmaceutical, cosmetics or production of clean energy (biodiesel, biogas, bioethanol)[1,2,8]. The wide diversity of compounds synthesized from different metabolic pathways of fresh and marine water algae provide important sources of fatty acids, steroids, carotenoids, polysaccharides, lectins, mycosporine-like amino acids, halogenated compounds, polyketides, toxins, agar agar, alginic acid and carrageenan[2]. It is also important to note that many of these compounds present very specific and complex chemical structures that makes often too difficult to reproduce them by hemi-synthesis or complete synthesis[10].

The microalgae sector is very dynamic throughout the world but it is still under-exploited in Morocco although that it has important potential due to its geographical location: the North Atlantic Ocean to the west and the Western Mediterranean Sea to the north. This is essentially what affirms a new study of the Institute of prospective economic world of the Mediterranean (IPEMED)[11]. Only 5 companies (Figure no 1) producing microalgae and derivatives have been identified in the Kingdom against 7 in Portugal, 15 in Spain and 21 in France. And if the market for *Spirulina* seems mature, the microalgae sector could be more promising with the experiments in the production of third generation biofuels currently conducted in the South[11,12]. This work is the first review presenting an overview of the main researches that have been conducted on microalgae in Morocco, the biodiversity of isolated species and their different areas of exploitation.

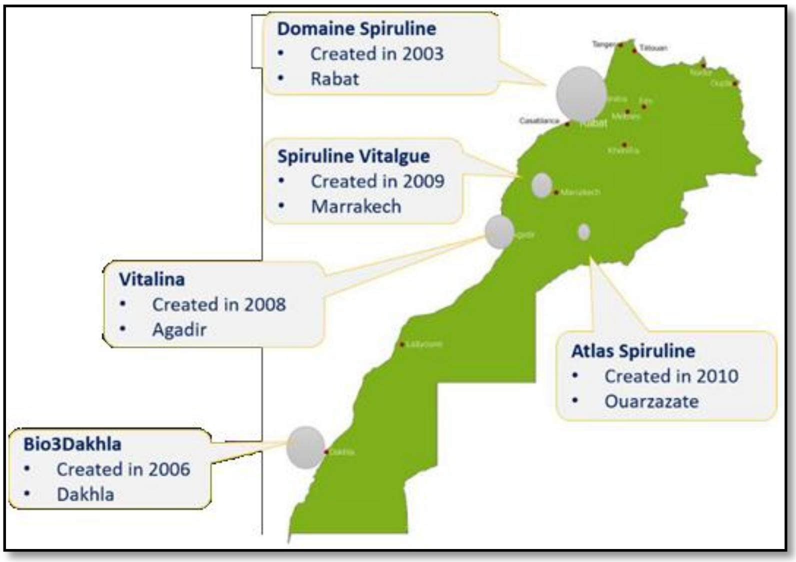

Figure no 1: Distribution of microalgal biomass produced in Morocco[12].

Microalgae For Biodiesel Production

Recently, there has been an increasing interest in microalgae due to the world energy crisis depleting world fossil-fuel reserves and increasing demands for renewable and clean energy. Microalgae are one of the most promising sources of biomass for biodiesel production due to their high photosynthetic efficiency, fast growth and high lipid content[13]. Transesterification using homogeneous and heterogeneous catalysts and in situ transesterification are possible methods to produce biofuel from microalgae lipids[14]. Analyses of thousands of microalgal species have shown tremendous difference in lipid content among different strains. Table no 1 illustrates this difference between some microalgae species. Moreover, the lipid content, lipid class and fatty acid composition fluctuate under different culture conditions for any one microalgal strain[15–18].

In Morocco, the screening of biodiesel producing microalgae has been done in some works[19–21]. The most interesting one was conducted by El Arroussi *et al.*[19]. They screened 57 marine microalgae isolated from the Moroccan coasts in order to find interesting candidates for a large scale biodiesel production. These microalgae belong to different genera (*Nannochloropsis*, *Chlorella*, *Dunaliella*, *Isochrysis*, *Phaeodactylium, Chaetoceros*, *Navicula*, and *Tetraselmis*). According to the criteria of growth and lipid rate, *Nannochloropsis sp*, *Dunaliella tertiolecta*, *Isochrysis sp* and *Tetraselmis* sp were selected as potential microalgae for biodiesel production. Selection criteria included also fatty acids profile, robustness and ease of culture. Moreover, they improved the potential of *Dunaliella tertiolecta for* biodiesel production by auxin treatment coupled to salt stress and by Nitrate reductase inhibition (Nitrogen stress)[20,22]. These treatments stimulate significantly biomass and lipid accumulation providing an increase in productivity and could eventually reduce cost of projects at commercial scale[20].

It is well known that an economically viable large-scale production of microalgae requires: i) warm temperature (25-30 °C), ii) proximity to sea water to not compete with agricultural resources, iii) availability of non-arable lands to avoid conflict with food production. Several regions of the south of Morocco (from Agadir to Dakhla) respond to these criteria. Culture systems can be raceways because of the low rainfall recorded in these areas over the year. In this local climate and using a productive microalgae strain, biomass productivity can reach 125-250 tones/ha/year with 94 000 liters of biofuels for each ha[23]. This represents a huge potential to exploit in order to develop microalgae-based biodiesel in Morocco. Nevertheless, no large-scale project has been carried out until know probably due to the lack of funding structure for pilot/industrial projects subvention[12].

Table no 1: Some microalgal species with relatively high lipid content[24]

Microalgae species	Lipid content (%, w/w)
Chlorella protothecoides	15–58
Chlorella sorokiniana	19–22
Chlorococcum sp.	19
Dunaliella salina	6–25
Ellipsoidion sp.	27
Nannochloropsis sp.	21-36
Nannochloropsis oculata	22-30
Neochloris oleoabundans	29–65
Pavlova salina	31
Pavlova lutheri	36
Phaeodactylum tricornutum	18–57
Scenedesmus sp.	20-21

Role Of Microalgae In Agriculture

Microalgae also have different applications in various areas of agriculture. They show positive impacts on soil quality for sustainable agriculture[25]. Microalgae increase the soil fertility by increasing the overall soil microbial activity and facilitating better microbial interactions, as they support the growth of beneficial microbes[26,27]. Recent studies revealed that microalgal biofertilizers play a significant role in seed germination, plant growth, improvement of soil fertility, nutritional value and crop yield[25]. Algal biofertilizers allowed also biocontrol of plant pathogens, besides their use as nutrient supplements[25]. Moreover, microalgae present promising potentials for the development of new products for plant growth stimulation [28,29].

In Morocco, few interesting works have been done regarding the use of microalgae in agriculture (Table no 2). Microalgae polysaccharide extracts tested in these works have shown positives effects on plants growth and crop yields, plants tolerance to salt stress and their defense mechanisms[30–32]. Five microalgae were screened: *Dunaliella salina, Chlorella vulgaris, Chlorella sorokiniana, Chlamydomonas reinhardtii and Spirulina platensis (Cynanobacteria*). *D. Salina* exopolysaccharides stimulated the growth of wheat and tomato and enhanced the tolerance of plants to salt stress of 3 and 6 g L^{-1} NaCl[30]. The total polysaccharides extract (TPE) from *Spirulina platensis* was applied to tomato and pepper plants. This treatment increased the plants size, the size and number of nodes per plant[33]. Moreover, the effect of the TPE treatment on roots weight was more pronounced in tomato plants (improvement of 230%) than pepper plants (improvement of 67%)[33].

While the crude polysaccharides extracted from the four green microalgae strains (*Chlorella vulgaris, Chlorella sorokiniana, Dunaliella salina and Chlamydomonas reinhardtii*) was injected into tomato plants to investigate their biostimulatory effects on different metabolic and biochemical pathways related to plant defense[31,32]. This treatment results showed that microalgae polysaccharides modulate the tomato lipid content by increasing polyunsaturated fatty acids content, stearic acid, palmitic acid, and very long chains fatty acids content[31,32]. Key defense enzymes such as β-1,3-glucanase, phenylalanine ammonia lyase and lipoxygenase were also stimulated with microalgae polysaccharides which suggest that these molecules present a promising renewable bio resource in the development of plant biostimulants.

Microalgae For Wastewater Treatment

Morocco as many countries of the Middle East and North Africa (MENA) are affected by water scarcity[34,35]. The reuse of treated wastewater for irrigation could be an alternative solution for a better water resources economy and could improve crop productivity and environmental sustainability[36]**.** Microalgae-based wastewater treatment technologies have shown many advantages in recent years to treat different water effluents (Municipal, agricultural or industrial)[37]. Microalgae are able to remove nitrogen and phosphorus (causing eutrophication) and to reduce both chemical and biochemical oxygen demand[38]. Microalgae cultures offer also an elegant solution to remove pathogens, some toxic organic compounds and toxic minerals such as lead, cadmium, mercury, scandium, tin, arsenic and bromine[1,34]. Therefore, it does not lead to secondary pollution. A large amount of algal species has been used in wastewater treatment. The most tolerant genera were found to be *Euglena, Oscillatoria, Chlamydomonas, Scenedesmus, Chlorella, Nitzschia, Navicula* and *Stigeoclonium*[34].

In Morocco, the main uses of microalgae in wastewater treatment processes have concerned the biosorption of metals (Cd, Cr, Cu and Zn) and the elimination of faecal coliforms, certain pathogens (*Salmonella, Vibrio cholerae*) and helminth eggs. The microalgae used in these works belong to the genera of *Chlamydomonas, Scenedesmus, Chlorella, Planothidium and Synechococcus* (cyanobacteria) (Table no 3). Mezrioui and Oudra[39] have reported other microalgae that have been used to treat wastewater by stabilization ponds under the arid climate of Marrakesh city (Morocco) (Table no 4).

Table no 2: Moroccan microalgae used in agriculture.

Microalgae	Crop	Effects	Reference
Dunaliella salina	Wheat *(Triticum aestivum* L.)	Stimulate germination and seedling growth in wheat under salt stress.	30
Spirulina platensis	Tomato (*Solanum lycopersium)* and pepper (*Capsicum annum)*	Increase plants size, roots weight and the size and number of nodes.	33
Dunaliella salina	Tomato *(Solanum lycopersicum)*	Enhance tomato tolerance to salt stress and stimulate its growth.	31
Chlorella vulgaris, Chlorella sorokiniana, Dunaliella salina, Chlamydomonas reinhardtii	Tomato *(Solanum lycopersicum)*	Biostimulate tomato defense	32

Table no 3: Microalgae used in wastewater treatment in Morocco and their effects

Microalgae	Source	Effects	Reference
Planothidium lanceolatum	Freshwater of Tensift River, Marrakech region	Biosorption of metals (Cd, Cu and Zn).	40
Chlorella sorokiniana *Synechococcus elongatus* *Synechocystis parvula*	Wastewater stabilization ponds of Marrakesh	Remove *E. coli* and *Vibrio cholerae.*	41
Chlamydomonas reinhardtii *Chlorella pyrenoïdosa* *Scenedesmus quadricauda*	Urban effluent of Fez city	- Remove phosphate and nitrogen; - Uptake of chromium and cadmium.	42
Microalgae mixture	Rabat city wastewater	- Biochemical oxygen demand, phosphate and nitrogen removal; - Removal of faecal coliforms, faecal *streptococcus*, pathogens (*Salmonella sp*) and helminth egg (nematodes) particularly those of the *Ascaris* and *Trichuris* genera.	43

Table no 4: Algal inventory in Marrakech wastewater stabilization ponds[39]

Division and order	Species
Euglenophyta	
Euglenales	*Euglena clavata* *Euglena pseudoviridis* *Euglena viridis* *Euglena pisciformis*
Chlorophyta	
Volvoccales	*Chlamydomonas* sp
Chlorococcales	*Chlorella sorokiniana* *Micractinium pusillum* *Coelastrum* sp
Chrysophyta	
Pennales	*Nitzschia umbonata*
Cyanophyta	
Oscillatoriales	*Oscillatoria irrigua* *Oscillatoria* sp
Chroococcales	*Synechococcus elongatus* *Synechocystis parvula*

Wastewater treatment with microalgae is particularly attractive and conform the new trends of wastewater treatment in future specially in some advanced world nations[34]. In Morocco, it should be noted that the semi-arid climate is very well suited to the growth of these photosynthetic microorganisms. However, more research is needed on the design and operation of microalgae-based wastewater treatment process to be more economical and more used.

Antibacterial Activity From Microalgae

Massive use of antibiotics has played an important role in the rapid emergence of antibiotic resistance[44]. Multidrug resistance is actually one of the greatest threats to global health, food security, and development worldwide[44]. Recently, new mechanisms of antibiotic resistance are appearing and compromising the ability to treat infections and have forced the efforts to find new antibacterial substances[45–47]. Numerous studies have shown that microalgae are a natural and important source of bioactive substances with proven antibacterial actions[48]. The screening for antimicrobial compounds from microalgae began in the 1950s[49]. However, in the last decade, large screening programs have been conducted to assess the potential antimicrobial activity of numerous microalgal species from distinct taxonomical groups originating from various areas[50]. The antimicrobial activity of microalgae has been attributed to different compounds including fatty acids[50], indoles, terpenes, acetogenins, phenols[51,52], pigments (phycobiliproteins)[53] or chlorophyll derivatives[54], polysaccharides[55], and also nanoparticles[56]. These various compounds present different mechanisms to inhibit bacterial growth[48]. Furthermore, the antibacterial efficiency of a compound can be different depending on the Gram type of the bacteria, the (G−) bacteria appeared to be more resistant to several antibiotic compounds than the (G+) bacteria)[48]. Last, the production of these compounds can change depending on the culture conditions and growth[57,58]. Their chemodiversity is also very important, this variability is species- but also isolate-dependent[48].

In Morocco, and despite the great capacity of microalgae to produce antimicrobial substances, they have only been studied in a single work carried out by Maadane *et al.*[59]. Nine marine microalgae screened from Moroccan coastlines were selected for this study. They were identified as: *Nannochloropsis gaditana*, *Dunaliella salina*, *Dunaliella* sp., *Phaedactylum tricornutum*, *Isochrysis* sp., *Navicula* sp., *Chaeotoceros* sp., *Chlorella* sp. and *Tetraselmis* sp. Their ethanolic extracts were evaluated against the bacteria: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the yeast *Candida albicans* and the fungus *Aspergillus niger* (Table no 5). The highest antibacterial activity was found in *Tetraselmis* sp. extract which exhibited an inhibitory effect against both the (G−) and the (G+) bacteria. Extracts from *Dunaliella salina*, *Nannochloropsis gaditana*, *Dunaliella* sp., *Phaeodactylum tricornutum* and *Isochrisis* sp. each showed inhibitory activity against *E. coli* or *P. aeruginosa*. However, the ethanolic extracts from *Chaetoceros* sp. and *Chlorella* sp. showed no effect under the applied experimental conditions. The extract of *N. gaditana* also inhibited *S. aureus* growth. The growth of *C. albicans* was inhibited by all the tested extracts while *Aspergillus niger* appeared to be insensitive[59]. These results show that marine microalgae are a potential natural source of bioactive compounds with antimicrobial activities, hence the need to continue the research and the isolation of new microalgae strains from Moroccan coastlines.

Table no 5: Antimicrobial activities of ethanolic extracts of nine Moroccan marine microalgae

Microalgae	*E. coli*	*P. aeruginosa*	*S. aureus*	*C. albicans*	*A. niger*
Tetraselmis sp	+	+	+	+	-
Dunaliella Salina	+	+	-	+	-
Nannochloropsis gaditana	+	+	+	+	-
Chlorella sp	-	-	-	+	-
Dunaliella sp.	+	+	+	+	-
Navicula sp.	+	+	+	+	-
Phaeodactylum tricornutum	+	+	+	+	-
Chaetoceros sp.	-	-	-	+	-
Isochrisis sp.	+	+	-	+	-

(-): absence of antimicrobial effect; (+): presence of antimicrobial effect.

II. Conclusion

Microalgal biotechnology has become a subject of research and study throughout the world for various fields simply by using their photosynthetic machinery to capture the light energy and use minimal nutrients to produce various bioactive metabolites. In Morocco, due to the mild climatic conditions and to its specific geographical position, microalgae present an interesting natural resource to promote locally. However, even if the potential remains important, the microalgae sector is still under-exploited in Morocco and *Spirulina* is the only microalgae produced. The use of microalgae in the fields of agriculture, food industry, medical, aquaculture and wastewater treatment requires more encouragement and funding to be developed in Morocco.

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