Advances in Environmental Biology, 9(20) Special 2015, Pages: 1-8

AENSI Journals

Advances in Environmental Biology

ISSN-1995-0756 EISSN-1998-1066

Journal home page: http://www.aensiweb.com/AEB/

Phycoremediation of Wastewaters and Potential Hydrocarbon from Microalgae: A Review

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ARTICLE INFO

Article history:
Received 25 July 2015
Accepted 30 August 2015
Available online 9 September 2015

Keywords:
Hydrocarbon; Phycoremediation; Wastewater; Microalgae; Heavy Metal; Biomass

ABSTRACT

Phycoremediation is one of the biological treatments that are considered sustainable and environmental friendly method to eliminate contamination in wastewater. Other than bio-transform pollutants in wastewater and microalgae also excellent source of hydrocarbon. Hydrocarbon from microalgal oil is considered sustainable since it is extracted from the biological plant. Nowadays, the increasing number of population and various types of industries in the world lead to the augmentation of wastewater disposal to the environment. Microalgal phycoremediation is one of the promising techniques that have high potential to assimilate the excessive pollutants in wastewater photosynthetically. Consequently, the discovery of new technologies to mitigate the adverse impact on the environment combined with sustainable hydrocarbon evaluation became one of the aims of this paper. Therefore, this article provides a mini review of wastewater phycoremediation studies and the potential of hydrocarbon extracted from microalgae.

To Cite This Article: Paran Gani, Norshuhaila Mohamed Sunar, Hazel Matias-Peralta, Ab Aziz Abdul Latiff, Umi Kalthsom Parjo, Abdul Rafiq Ab. Razak, Phycoremediation of Wastewaters and Potential Hydrocarbon from Microalgae: A Review. ***Adv. Environ. Biol., 9(20),*** *1-8, 2015*

INTRODUCTION

Nowadays, selection of wastewater treatment method is one of the most interesting topics among the researcher either conventional, bioremediation or advanced method. Phycoremediation is a bioremediation technique in wastewater treatment that utilizes microorganism such as microalgae. According to Ahmad *et al*. [1] the use of algae to treat wastewater has been in practice for over 40 years. The first use of this discovery was introduced by Oswald [2]. In previous years, the microalgae have attracted much attention as an alternative to the conventional treatment method. This is primarily because, microalgae wastewater treatment is eco-friendly and offers the advantages of a cost effective way of pollutant load removal [3]. Recently, phycoremediation technology utilizing microalgae has been combined with hydrocarbon production [4, 5]. Besides that, technology towards producing hydrocarbon from microalgae has become very popular. Microalgae clearly presented few advantages, among which is the higher biomass production than terrestrial plants. In addition, growing this biomass does not require high-quality agricultural lands [6]. Moreover, microalgae need a lower rate of water renewal if compared to the terrestrial crop where microalgae able to be cultivated in brackish water. Microalgae require only sunlight and few simple and non-expensive nutrients such as nitrogen, phosphorus, and carbon [7]. To top all the above-mentioned advantages, hydrocarbon produced by microalgal biomass is expected to offer a variety of income opportunities and a potential source of clean fuel that may reduce GHG emissions. Hence, boosting the decarbonization of transportation fuels and increasing the security of energy supply [8]. Consequently, the objective of this article is to provide a mini review of studies utilizing microalgae for hydrocarbon production after phycoremediation of wastewaters.

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Literature review:

Phycoremediation:

Phycoremediation could be defined in a wider meaning as use of microalgae or macroalgae for removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO_2 from wastewater with concomitant biomass propagation [9]. There are several stages of treating wastewater, industrial effluents and solid wastes using microalgae aerobically as well as anaerobically. Bioremediation usually depends on various laws established but also able to rely on the assessment of human health and ecological risks where there is a lack of established legal standards [10]. As introduced by John [11] where the term of phycoremediation was referred to the remediation carried out by algae. Oswald [12] believes that the use of microalgae for the treatment of municipal wastewater will be a topic of research and development that will attract public attention for several years to come. Extensive works have been conducted to explore the feasibility of using microalgae for wastewater treatment, especially for the removal of nitrogen and phosphorus from effluents [13 - 16]. Reduction and removal of nitrogen and phosphorus by microalgae bring a lot of benefits particularly in alleviating the deteriorating environmental quality. Studies also showed the reduction of several heavy metals contaminations by microalgae [17].

In order to improve the understanding about relationship between microalgae and bacteria in promoting the removal of nutrients and harzardous pollutant, Figure 1 below describe the biological combination treatment where microalgae growing in wastewater are associated with bacteria. These associations due to the bacteria constitute an important sources of CO_2 for algae growth. After that, organic compound and O_2 realesed by algae can be assimilated by bacteria to boost their population. Then, cooperation between microalgae and bacteria resulted the biotransformation of pollutants in wastewater from toxic to non or less toxi. At the same time, microalgae biomass definitely be able to maximized [17].

Therefore, Rawat *et al*. [10] briefly describes the mechanisms involved and apply the nutrient removal and reduction of industrial wastewater by microalgae is almost the same as those experienced by domestic wastewater treatment. Phycoremediation comprises several applications: (i) nutrient removal from municipal wastewater and effluents rich in organic matter; (ii) nutrient and xenobiotic compounds removal with the aid of algae-based sorbents; (iii) treatment of acidic and metal wastewaters; (iv) $CO2_2$ sequestration; (v) transformation and degradation of xenobiotics; and (vi) detection of toxic compounds with the aid of algae-based biosensors. Nutrient removal with the help of microalgae compares very favorably to other conventional technologies [10].

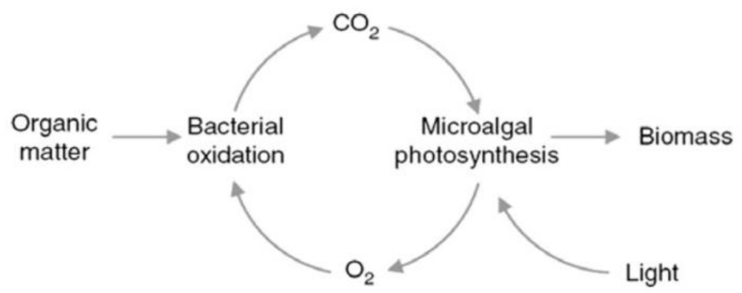

Fig. 1.0: Principle of photosynthetic oxygenation in BOD removal [17].

Advantages of Phycoremediation:

There are many known advantages of phycoremediation in the process of treating wastewater (e.g. Rawat *et al*. [10]; Abeliovich, [18]; Narro, [19]; Pinto *et al*. [20]; Tilzer, [21]; Cerniglia *et al*. [22]; Cerniglia *et al*. [23]; Pinto *et al*. [24]; Lima *et al*. [25]. Furthermore, Sivasubramaniam [26] listed down few more benefits of using microalgae in treating wastewater pollution:

1 Phycoremediation is a cost-effective, eco-friendly and a safe process.
2 The microalgae employed are non-pathogenic photosynthetic organisms and they do not produce any toxic substances.
3 Phycoremediation effectively reduce nutrient load thereby reducing total dissolved solid.
4 Phycoremediation reduces sludge formation to a enormous extent.
5 Phycoremediation increase dissolved oxygen levels through photosynthetic activity.
6 Phycoremediation keeps the bacterial population under control.
7 Algal growth in the effluent also removes waste CO_2 from the air thereby contributing to the reduction of greenhouse gasses.
8 The algal biomass has high nutrient value and can be suitable as a live feed for aquaculture.
9 The algal biomass could also be used as a Bio-fertilizer and in EM (Effective Microbes).

10 Conventional chemical treatment of effluent results in concentrating the toxic waste in the form of sludge and requires landfill. Whereas phycoremediation detoxifies and removes it forever.
11 Minimal odour compared to conventional methods of treatment.
12 Simple operation and maintenance.
13 Construction and operation costs are typically less than half those of mechanical treatment plants (e.g. activated sludge, sequencing batch reactors).
14 Sustainable treatment solution with significant potential for energy and nutrient recovery.

Application of Phycoremediation in Wastewaters Treatment:

Nowadays, microalgae have become an important or significant microorganism for biological purification and treatment of wastewater. This may due to them can accumulate and assimilate plant nutrients, heavy metal, pesticides, organic and inorganic pollutants and radioactive matter in their unicellular cells [27]. Microalgae cultivation combined with wastewater treatment system offer more simple, convenient and economical technology or technique as compared to another environmental alternative system [28]. Moreover, photosynthesis can be effectively exploited to generate oxygen from wastewater phycoremediation. Then, the choice of microalgae species used in wastewater pollutant biotransform is determined by their robustness against the contamination and also growth efficiency. Some of the selection of microalgae species and wastewater treatment are summarized in Table 1.

As shown in Table 1, Sahu [27] studied the Chlorella vulgaris in organic and inorganic pollutant reduction using sewage from the treatment plant. His analysis revealed that 70% of BOD, 66% of COD, 71% of TN, 67% of phosphorus, 54% of volatile solid and 51% of dissolved solid was reduced. Meanwhile, industrial wastewaters had been treated using microalgal bacterial flocs was done by Van Den Hende *et al*. [29] found that a significant removal of turbidity, BOD, TCOD, TOC, TC, TN and TP are 96%, 87%, 80%, 71%, 48%, 58% and 8%, respectively. They also observed the final effluent DO was 6.06mg/L and the average pH was a bit alkalinity. Azarpira *et al*. [30] compared two species of cynobacteria namely *Oscillatoria limosa* and *Nostoc commune* in the removal of nutrients using polluted river water from Mula-Mutha, Pune. The average reduction efficiency was between 84% - 98%. Amongst the selected cynobacteria, *Oscillatoria limosa* was the best as compare to *Nostoc commune*. Consequently, both algal also have superb potential for nitrogen fixation and biomass for paddy cultivation. In bioremediation of primary treated wastewater using *Chlorella minutissima, Nostoc* and *Scenedesmus* was attempted by Sharma & Khan [31]. The end result showed that these algae were very efficient in reduction of the physiochemical parameter in sewage wastewater.

Further, they observed that *Chlorella* had the best phycoremediation that can remove of TDS 97%, Nitrogen 90%, Phosphorus 70%, BOD 95% and COD 90%. Despite that, *Scenedesmus obliquus* study by Ji *et al*. [32] in piggery wastewater treatment was capable to remove TN around 23-58% and TP 48-69% only and they suggested *Scenedesmus obliquus* was a promising candidate for environmental friendly bioenergy sources. In a different study, Kshirsagar [33] examined *Chlorella vulgaris* and *Scenedesmus quardricauda* in domestic wastewater. Thus, found that *Chlorella* effectively remove of COD 80.04%, BOD 70.91%, nitrate 78.08% and phosphate 62.73% while *Scenedsmus* able to remove COD 70.97%, BOD 89.21%, nitrate 70.32% and phosphate 81.34% on the 15^{th} days of cultivation. In another microalgae species and different type of wastewater, Riaño *et al*. [34] conducted an experimental investigation on *Oocyctic* sp. in fish processing wastewater treatment. Two photobioreactor inoculated with microalgae (*Oocystis* sp.) at 23°C and 31°C, respectively. They measured approximately 70% of TCOD and phosphate removal was achieved regardless to the temperature.

Table 1.0: Application of Microalgae in Wastewaters

Phycoremediation study summary	Study location	References
Phycoremediation of greywater and dairy wastewater by *Botryococcus* sp. for physiochemical parameters removal.	Johor, Malaysia	[39, 40]
Application of *Chlorella vulgaris* for reduction of organic and inorganic pollutant in sewage treatment plant wastewater.	Kombolcha, Ethiopia	[27]
Application of microalgal bacterial flocs for industrial wastewater treatment using sequencing batch reactors.	Ghent, Belgium	[29]
Phycoremediation of municipal wastewater using *Oscillatoria* and *Nostoc commune* obtained from Mula-Mutha river.	Pune, India	[30]
Bioremediation of primary treated wastewater using different types of microalgae (*Chlorella minutissima,* BGA *Nostoc* and *Scenedesmus*).	New Delhi, India	[31]
Piggery wastewater treatment using isolated *Scenedesmus obliquus* obtained from municipal wastewater effluent.	Wonju, South Korea	[32]
Domestic wastewater bioremediation using microalgae *Chlorella vulgaris* and *Scenedesmus quadricauda* for physiochemical reduction.	Pune, India	[33]
Application of *Chlorella vulgaris* in wastewater treatment for drainage solution from commercial greenhouse tomato cultivation.	Alnarp, Sweden	[41
Phycoremediation of sewage drainage using *Chlorella vulgaris, Rhizoclonium hieroglyphocum* and mixed algal.	Lahore, Pakistan	[1]

pH analysis in tannery industry wastewater when cultivated with *Chlorella* sp. for treatment purpose	Coimbatore, India	[42]
Cultivation of *Botryococcus braunii* in greywater for physiochemical and microbiological parameter analysis.	Pondicherry, India	[38]
Phycoremediation of dairy effluent collected from Madavaram dairy plant by *Nostoc* sp. for 15 days under open condition.	Chennai, India	[43]
Treatment of leather-processing chemical wastewater using single isolated green microalgae *Chlorella vulgaris*.	Tamil Nadu, India	[44]
Treatment of fish processing wastewater using *Oocystis* sp. in photobioreactors experimental.	Valladolid, Spain	[45]
Collected open drain wastewater treatment by *Cloacal* algae cultivation for physiochemical and heavy metal removal.	Agra, India	[35]
The use of *Chlorella vulgaris* obtained from Malaya Algae Culture collection for bioremediation of textile wastewater collected from Senawang Industrial Estate, Negeri Sembilan.	Kuala Lumpur, Malaysia	[46]
Application of phycoremediation technique using *Chlorella vulgaris*, *Synechocystis salina* and *Gloeocapsa gelatinosa* in industrial polluted water.	Kerala, India	[47]
Growth of Botryococcus braunii using urban wastewater from secondary treatment plant for phycoremediation purpose.	Jaen, Spain	[37]
Physiochemical analysis of Thermal power station effluents treated with *Pithophora* sp.	Chennai, India	[36]
Phycoremediation of domestic wastewater and industry wastewater using *Botryococcus* sp. coupled with hydrocarbon evaluation.	Johor, Malaysia	Currently investigation carried out by the authors

Previously, in 2011, Sengar *et al*. [35] revealed that *Cloacal* algae could change the pH of open drain wastewater from 8.1 to 7.1 and also increase the DO about 87.5% on the 25^{th} days of cultivation. Bioremediation, of thermal wastewater by *Pithosphora* sp. also showed a positive result that it can remove the physiochemical from 32% to 92% as done by Murugesan & Dhamotharan [36].

Based on the microalgae selection in wastewater treatment where many microalgae have a high potential for treating wastewater. In most of the microalgae as listed in Table 1, there are quite few research studies on *Botryococcus* sp. in wastewater phycoremediation using domestic wastewater and industry wastewater. Furthermore, *Botryococcus* sp. only has been adopted in urban wastewater [37] and greywater [38] only. But recently, Gani *et al*. [39, 40] had done phycoremediation studies by treating the greywater and dairy wastewater, respectively using 1000 cell/mL as starting initial concentration. Our result found that *Botryococcus* sp. be able to reduce the physicochemical up to 73.3% for BOD in dairy wastewater meanwhile remove 88% for COD in greywater. Then, realizing the gap in the extant literature, more research is needed for *Botryococcus* sp. in treating wastewater such as domestic and industrial combined with hydrocarbon production.

Bioremediation of Heavy Metal by Microalgae in Wastewater:

According to Chekroun & Baghour [48], heavy metal is a pollutant that considered to be a significant environmental problem related to human health. The contamination of water by toxic metals and organic pollutants recently increased due to anthropogenic activity. Thus, bioremediation technique to assimilate that toxic has a high potential to be applied in wastewater treatment. Bioremediation is a process of using specific microorganisms to transform hazardous contaminations in water to nonhazardous waste products [49]. In 2012, Dwivedi also described there are two steps involved in the assimilation of heavy metals. First, the metals are adsorbed over the cell very quickly called physical adsorption. Next, these metals are assimilated slowly into the cytoplasm in a process named chemisorptions. However, absorption of heavy metal depends on the other parameter such as pH. As highlighted by Dwivedi [49], surface charge studies showed that the availability of free sites depended on pH. With increasing pH, the surface charged sites of calcium alginate became more negative, then the uptake of metal increased with increasing pH.

Therefore, Table 2 shows the selection of microalgae in bio-remediate some of the heavy metals ions done by previous researchers. Worku & Sahu [28] cultured *Synechocystis salina* in groundwater to reduce the heavy metals and total hardness within 15 days of treatment. At the end of the treatment day, *Synechocystis salina* be able to remove of Cr 60%, Fe 66%, Ni 70%, Hg 77%, Ca^{2+} 65%, Mg^{2+} and total hardness 78%. Meanwhile, Kumar *et al*. [50] had demonstrated to remove Zinc using immobilized and powder form from *Chlorella marina*. They found that the highest removal fall to the powder form of 97% compared to immobilized of 55.3%. At the same time, the optimum pH for the heavy metal adsorption is at pH 8. In bioremediation of industrial wastewater, Soeprobowati & Hariyati [51] used *Porphyridium cruentum* isolated from brackish water to assimilate the Pb, Cd, Cu and Cr. During the experimental, pH, temperature, salinity and light were maintained to be on 7-8, 28-32°C, 32-34 ppt and 4200 lux, respectively. Thus, this red microalga was able to reduce Cu of 92 % from the wastewater. In a different study, Ajayan & Selvaraju [52] examined two strain of microalgae; *Chlorella pyrenoidosa* and *Scenedesmus* sp. in tannery effluent. As mentioned in Table 2, they analyzed that the highest removal using both microalgae were Copper, 77% and 79.2%, respectively.

Whereas Krustok *et al*. [53] were applying the Indigenous microalgae in wastewater collected from WWTP in Vasteras. Their finding was showing that this microalga very effective in removing of Barium 91.2 % and Iron 94.6 %. In summary, most of microalgae species, as listed in Table 2, have their advantages in bioremediation of heavy metal in water. Other than nutrient (Phosphorus and Nitrogen), microalgae also need a heavy metal element to build their cell, for example, iron and chromium [49]. Also, a major advantage using microalgae in bioremediation is that this process under the light condition and does not need oxygen. Instead they absorb CO_2 and release O_2. However, to the best author's knowledge, no report has been found so far using *Botryococcus* sp. in bioremediation of heavy metals in wastewater. To address this gap, the application of *Botryococcus* sp. in sewage treatment was the motivation behind the present project.

Table 2.0: Heavy Metals bioremediation by microalgae

Microalgae species	Heavy Metal Study		References
	Metals	Removal (%)	
Synechocystis salina	Cromium (Cr)	60	[28]
	Iron (Fe)	66	
	Nickel (Ni)	70	
	Mercury (Hg)	77	
	Calcium (Ca^{2+})	65	
	Magnesium (Mg^{2+})	63	
	Total Hardness	78	
Chlorella marina	Zinc, Zn (Powder)	97	[50]
	Zinc, Zn (Immobilized)	55.3	
Porphyridium cruentum (S.F. Gray)	Copper (Cu)	92	[51]
Chlorella pyrenoidosa	Cromium (Cr)	52.8	[52]
	Copper (Cu)	77.1	
	Lead (Pb)	43.8	
	Zinc (Zn)	68.9	
Scenedesmus sp	Cromium (Cr)	52	
	Copper (Cu)	79.2	
	Lead (Pb)	47.8	
	Zinc (Zn)	66	
Indigenous microalgae	Barium (Ba)	91.2	[53]
	Iron (Fe)	94.6	

Microalgae and Hydrocarbon Production:

When researcher discusses about hydrocarbon, it is referring to the production of lipid content and lipid productivity. As discussed earlier, the hydrocarbon is a combination of two elements namely hydrogen and carbon. The composition of hydrocarbon exists in many products and materials. Currently, the popular sources of hydrocarbon come from fossil fuel. Well known which is the hydrocarbon from petroleum dwelling day by day. It also considered unsustainable and gave negative impact to our environment. However, it is different from hydrocarbon made of the biological plant. Since the microalgae are photosynthesis organisms and the hydrocarbon obtained called as bio hydrocarbon. Hydrocarbon from biological plant expected given the discovery new renewable energy more valuable. Nowadays, the production of hydrocarbon by previous author normally used for biofuel production. Biofuel can be categorized as primary and secondary.

Table 3.0: Comparison of biodiesel sources [59, 60]

No.	Sources	Oil yield L/Ha
1	Corn	172
2	Soybean	446
3	Canola	1190
4	Jatropha	1892
5	Coconut	2689
6	Palm oil	5950
7	Microalgae (70% oil in biomass)	136900
8	Microalgae (30% oil in biomass)	58700

Then biodiesel is a secondary as the 3^{rd} generation of biofuel from microalgae [54]. The potential of microalgae as a source of renewable energy has received considerable interest, but if microalgae biofuel production is to be economically viable and sustainable, further optimization of mass culture conditions are needed [55]. As shown in Table 3, microalgae appear to be the only source of biofuel that has the potential to displace completely fossil diesel. Unlike other oil crops, microalgae grow extremely rapidly and many are exceedingly rich in oil. Oil content in microalgae can achieve 30-70% by weight of dry biomass. The production of various types of lipids, hydrocarbons and other oil by microalgae usually depends on the species of microalgae used as most microalgae has its own special characteristics [56 – 59]. Most importantly, they do not compete with food crop and can be produced using non-arable land, wastewater in the bioreactor [59].

Conclusion:

To meet the environmental regulation, all types of wastewater need to be treated before its discharge to the river. Wastewater treatment is the important process to remove or minimize the contaminant. Therefore, the potential of microalgae to be used to treat the wastewater to remove the chemical and organic contaminants, heavy metal and pathogen due to the wastewater rich in CO_2 moreover, it is provided conducive growth medium for microalgae to assimilate those contaminations. The resulting biomass is energy rich which can be further processed to make biofuel, biodiesel, and other bio-hydrocarbon. Other than that, algae biomass also can be used to obtained product called as a bio-based product such as bio-plastic, fertilizer, micro-beads, animal food and many more.

ACKNOWLEDGEMENT

The authors are grateful for the support of any parties that are involved in this project, especially University Tun Hussein Onn of Malaysia for providing the equipment research facilities to carry out this project. Special thanks to MyBrain15 Grant Scheme for the research sponsorship and other research team members.

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