Punjab Univ. J. Zool., Vol. 31 (2), pp. 307-320, 2016

ISSN 1016-1597 (Print)
ISSN 2313-8556 (online)

Review Article

Microalgal-bacterial consortium: a cost-effective approach of wastewater treatment in Pakistan

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(**Article history:** Received: October 31, 2015; Revised: December 09, 2016)

Abstract

Wastewater treatment using microalgae is an environmental friendly practice, involving various interactions among the micro and macro fauna and flora of treatment plant. The use of algal-bacterial consortia for wastewater treatments has proved more effective in biomass production, nutrient cycling and bioremediation of organic pollutants, heavy metals and many other contaminants. The biodegradation approach of involving consortium might attain self-sustaining level and may prove technically cheaper and advance technology. It will finally help in dealing the dual mission of producing valuable metabolites and pollutants/nutrients removal from wastes/wastewaters. Agricultural wastes/residues generated abundantly in energy deficient countries like Pakistan, having enormous energy potential, are not utilized efficiently and considered as wastes only. The present review focuses on the current research on algal-bacterial consortia and the construction of consortia by keeping in view the environmental conditions of country, suitable for microalgal growth to deal with pollution control and production of animal/fish feed and other valuable metabolites.

Keywords: microalgal-bacteria consortium; bioremediation; nutrient removal; CO_2 sequestration

To cite this article: MANZOOR, M., MA, R., SHAKIR, H.A., TABSSUM, F. AND QAZI, J.I.,. 2016. Microalgal-bacterial consortium: a cost effective approach of wastewater treatment in Pakistan. *Punjab Univ. J. Zool.,* **31**(2): 307-320.

INTRODUCTION

Due to the increased environmental threats and issues of our health, there is need to treat wastewater before being discharged into the environment. Many biotechnological techniques have been utilized to treat pollution and wastewaters in the past as well as today. Microalgae/cyanobacteria and bacteria consortium is new amongst them. Naturally Cyanobacteria and microalgae are found in association with other anaerobic and aerobic microbes. These bacterial associations may enhance or declines the algal growth (Fukami *et al*., 1997). Park *et al*. (2008) reported that even old algal cultures also develop symbiotic associations with bacteria. Oxygen produced by algal/microalgal photosynthesis is utilized by bacteria to degrade the organic matter, whereas CO_2 generated during this process helps in completing photosynthetic process/cycle. Such a type of symbiotic cooperation/relationships of heterotrophic bacteria and phototrophic microalgae are the basis of removal of BOD from wastewater treatment units and could be helpful in dealing with environmental contaminants (Oswald *et al*., 1953; Safonova *et al*., 1999; Chaillan *et al*., 2006). Therefore, algal-bacterial consortium has attained great importance in past few years as process is sustainable and cost-effective for treatment of animal, municipal and industrial wastewaters (De-Bashan *et al*., 2002; Olguin, 2003; Munoz *et al*., 2005; Aslan and Kapdan, 2006; Munoz and Guieysse, 2006; Zhang *et al*., 2009).

Microalgae: biodiesel feedstock

Microalgae are usually found in brackish, marine and freshwater, on soil surface and sometimes also in symbiotic relationship with other organisms. They are autotrophic and microscopic organisms. Currently microalgae are being used as non-food feed stocks for production of biodiesel due to the fossil fuels

25-PUJZ-51021011/16/0307-0320

*Corresponding author: maleeha.manzoor@yahoo.com depletion and discharge of greenhouse gases (Damiani *et al*., 2010; Xiong *et al*., 2010). About 50,000 microalgal species have been discovered yet and most of them have lipid contents up to 20-50% of their biomass which makes them more suitable for biodiesel production (Brennan and Owende, 2010). Microalgae are able to produce different biofuels *e.g.* biodiesel, biohydrogen, bio-oil and bio-syngas (Li *et al*., 2008a).

In comparison of plant derived oils, microalgal derived oils have many advantages. Microalgae have short carbon period of few days only and grow rapidly. They have a high biomass yield and increased photosynthetic efficiency than that of land plants (Tredici, 2010). Microalgae have higher photosynthetic efficiency which ranges from 3 to 8% as compared to that of the terrestrial plants, which is only up to 0.5% (Lardon *et al*., 2009).

Many algal species are able to convert CO_2, nutrients and sunlight into lipids, proteins and carbohydrates with increased growth rates. Their biomass enhanced growth rates can show increase upto five times in a day with addition of certain nutrients and proper aeration (Renaud *et al*., 1999; Aslan and Kapdan, 2006). Microalgae can be harvested on daily basis and are used as feedstocks for food, fish/animal feeds, chemicals and biofuels (Breuer *et al*., 2012).

Microalgae can also be used as a feedstock for the production of butanol and ethanol etc (Stephens *et al*., 2010). In addition they have least competition for land than that of the oil crops and are able to grow anywhere, where plenty of water, sunlight and the nutrients are available. Microalgae can be cultivated in wastewaters instead of freshwaters or in their specified nutrient media.

They are cultivated all round the year, therefore the oil production exceeds the yield of best oil producing crops. Along with the oil production many microalgal species produces other compounds with important applications, including fats, polyunsaturated fatty acids (PFAs), pigments, dyes, sugars, high-value compounds, antioxidants and biomass (Li *et al*., 2008a, b; Raja *et al*., 2008).

Therefore microalgae have the ability to revolutionize many areas of biotechnology including nutritions, cosmetics, biofuels, pharmaceuticals, food additives, pollution control and aquaculture/animal feed (Raja *et al*., 2008; Rosenberg *et al*., 2008).

Microalgal growth on wastes

Microalgae can also be cultivated in wastewaters instead of the specific media or in freshwaters. They are cultivated all round the year; therefore the oil production exceeds the yield of best oil producing crops. Microalgae cultures offers an interesting step for waste water treatments which are rich in micro and macro nutrients (Schenk *et al*., 2008; Park *et al*., 2010; Pittman *et al*., 2011). Therefore, they can be applied for tertiary biotreatment of wastewaters coupled with production of valuable biomass (Christenson and Sims, 2011). They are capable to remove phosphorus, nitrogen (major cause of eutrophication) and toxic metals from waste waters (Oswald, 2003; Graham *et al*., 2009; Christenson and Sims, 2011; Pittman *et al*., 2011). There are several successful reports of algal growth on industrial waste waters, agricultural waste waters and municipal waste waters (Mulbry *et al*., 2008; Mulbry *et al*., 2009; Chinnasamy *et al*., 2010; Chi *et al*., 2011; Li *et al*., 2011; Markou and Georgakakis, 2011).

For the removal of phosphorus and nitrogen from municipal wastewater, microalgal cultivation is being most extensively applied (Bhatnagar *et al*., 2010; Ruiz-Marin *et al*., 2010; Chi *et al*., 2011; Li *et al*., 2011). Another major source of effluents capable of supporting algal growth is agricultural wastewaters, livestock production being the main source. Large scale livestock operational set up which emerged during the past few decades have resulted accumulation of high concentration of nutrients in the affected areas. Industrial wastewaters from the carpet industry have been used as an algal growth medium for the removal of heavy metals, nitrogen and phosphorus (Chinnasamy *et al*., 2010). There is wide scope of recruiting select microalgal species for cultivation in wastewaters purposefully for remediating the effluents and production of valuable fuels. For example, *C. mexicana* has proved to be one of the promising candidate for high efficient biodiesel production and wastewater treatment (Abou-Shanab *et al*., 2013).

Microalgae are also reported to have high capacities of metal uptakes such as certain microalgal species like those of the genera *Pleurochrysiscarterae*, *Chlorella* and *Botrycoccus* grow well in the untreated industrial wastewaters (Chinnasamy *et al*., 2010). *Botrycoccus braunii* shows effective growth and efficient uptake of NO_3 when grown in piggery wastewaters (An *et al*., 2003). The algal species *Ulothrix* sp., *Microsporawilleana* sp. and *Rhizocolonium hieroglyphicum* showed potential growth when cultivated in wastewaters of dairy industries (Mulbry and Wilkie, 2001; Wilkie and Mulbry, 2002; Mulbry *et al.,* 2008). *Micractinium* sp., *Actinastrum* sp*., Dictyosphaerium* sp*., Pediastrum* sp., and *Coelastrum* sp*.,* are some of the microalgal species which dominate in wastewaters treatment (high rate algal ponds) HRAPs and form large colonies which are easy to harvest (Beneman, 1996; Park *et al*., 2010). *Chlorella* sp. and *Scenedesmus* sp. are known for accumulation of nutrients from the wastewaters and are particularly tolerant to the sewage effluents (Lau *et al*., 1995; Gonzalez *et al*., 1997; Masseret *et al*., 2000; Aslan and Kapdan, 2006; Shi *et al*., 2007; Ruiz-Marin *et al*., 2010; Wang *et al*., 2010). *Scenedemus obliquus* has a high potential for lipid production and CO_2 capturing (Mandal and Mallick, 2011) and show better growth in the municipal wastewaters (Ruiz-Marin *et al*., 2010). Microalgae are not being utilized frequently due to their high production costs (Khoshmanesh *et al*., 1996; Can˜izares -Villanueva, 2000). A significant cost reduction process can also be achieved by using waste based media (Medina and Neis, 2007). Therefore algal-bacterial consortium is advantageous as it makes the wastewater treatment process more profitable.

Algae-bacterial Consortium

Fungal and bacterial biomass from wastewaters or industrial fermentations is being utilized in biosorption processes because they are abundant and cheap/less expensive. The idea of treating industrial and domestic wastewaters by using algal-bacterial culture/consortium is gaining attention the past few years (Garcia *et al*., 2000; De-Bashan *et al*., 2002; Munoz *et al*., 2005; Medina and Neis, 2007; Bordel *et al*., 2009). The microbial consortium driven wastewater remediation is the method of choice in regions with year-round high solar radiations as the process might be powered by solar energy (Oswald, 2003). Under these illuminated conditions, algae uptake the nutrients and release O_2 which can be utilized by aerobic bacteria as electron acceptor, for degradation of organic matter. At the same time algae also consume CO_2 produced by the bacteria and helps in CO_2 sequestration while yielding intracellular lipids in the microalgae (Oswald and Gotaas, 1957; Oswald, 1988 Munoz and Guieysse, 2006) (Fig.1).

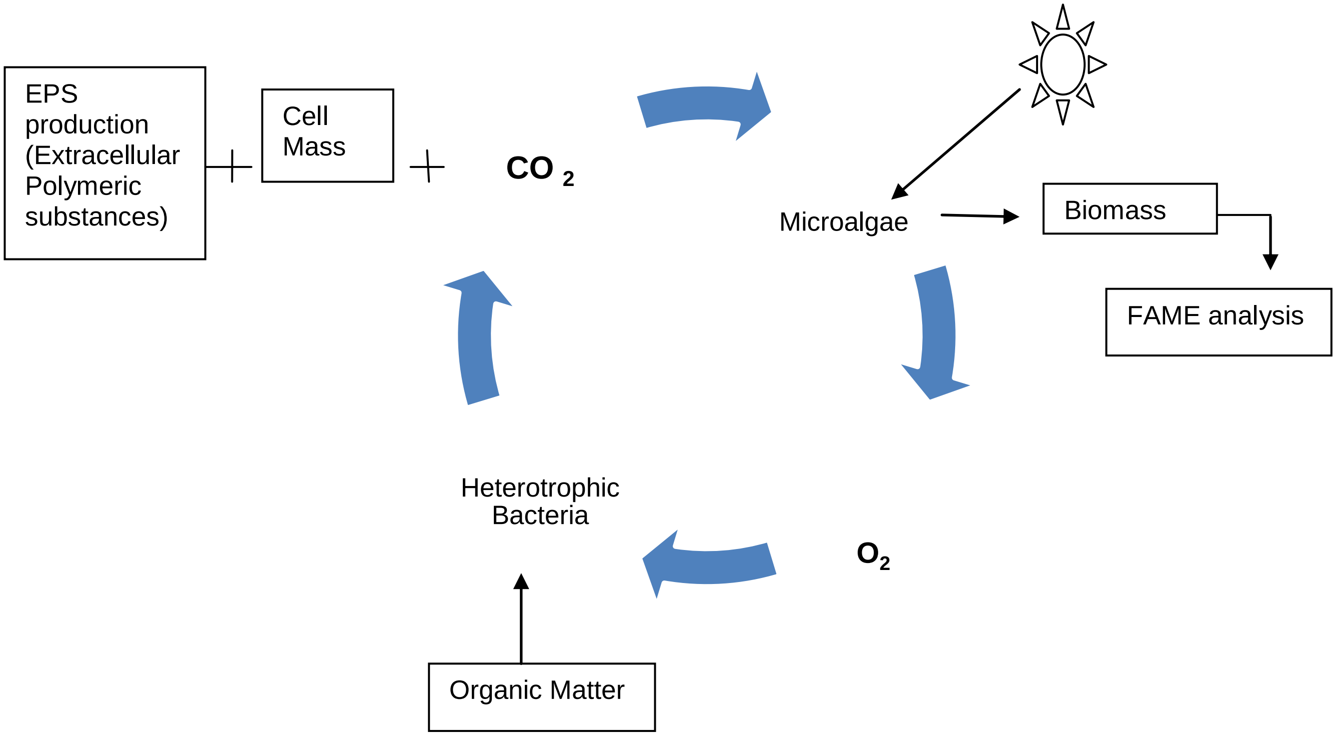


Figure 1: Schematic Layout of symbiotic principle of algal-bacterial culture for wastewater treatment

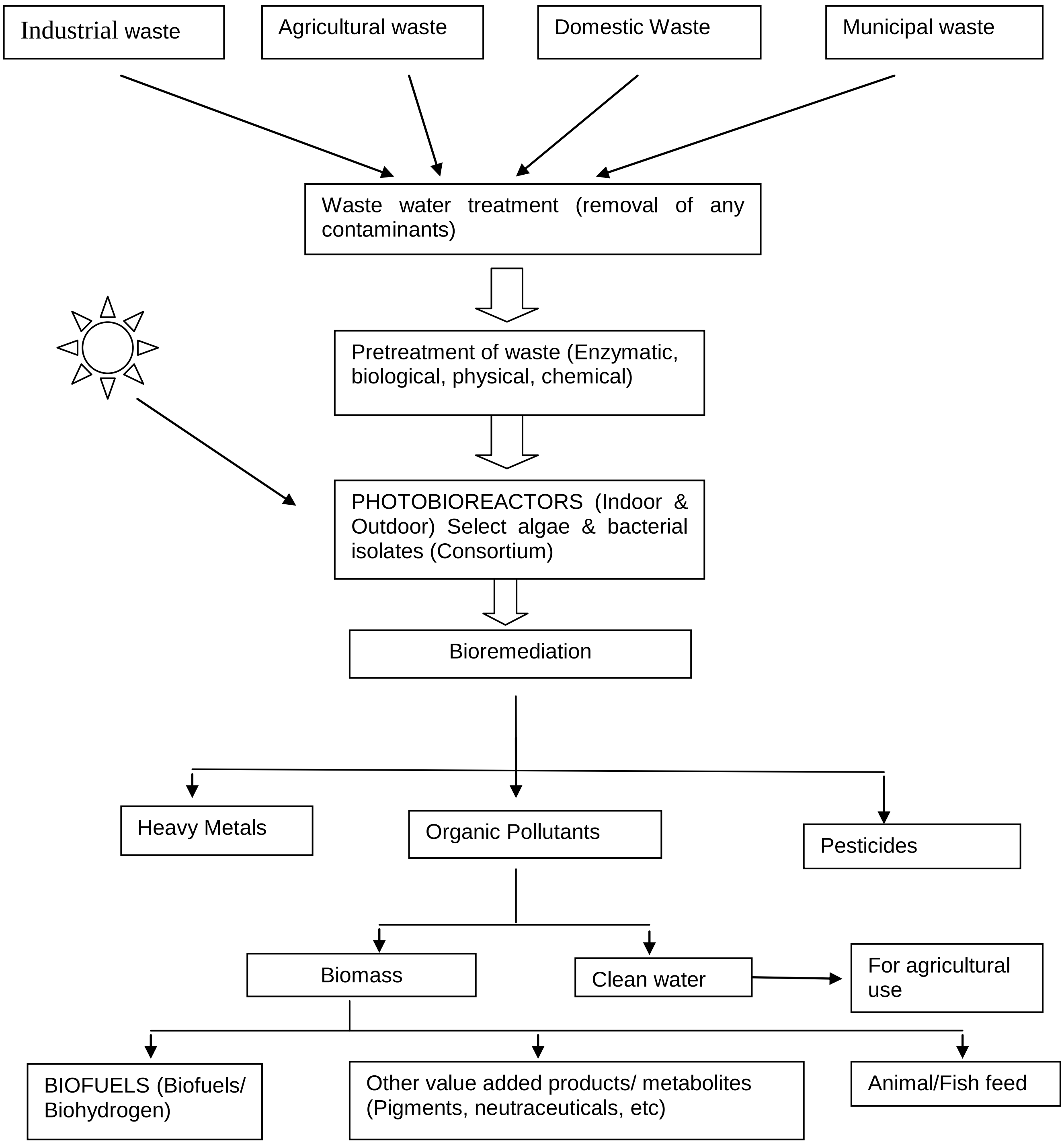


Figure 2: Sunlight mediated eco-friendly algae/bacterial consortium

Nitrogen and phosphorus are also efficiently removed from the wastewaters by accumulating into algal-bacterial biomass (Wang *et al*., 2010). Another algal-bacterial consortia advantage is that the biomass has enhanced settle ability as compared to algal biomass only, which helps in trimming down the harvesting cost of biomass (Medina and Neis, 2007). In this sense microalgal-bacterial systems have been gaining special attention in last year's. In these consortia, bacteria produce CO_2 which is the basic requirement for algal growth whereas microalgae produce O_2 which is utilized by bacteria. It is reported in literature that various types of interactions occur in algal-bacterial systems (Cole, 1982). Croft *et al*. (2006)

suggested that bacterial activity is enhanced due to the release of extracellular compounds by algae whereas bacteria were coupled with proficient exchange of substrates. Similarly bacteria are able to increase algal growth by utilizing O_2 of the medium by removing algal toxic compounds being released in the medium, by releasing growth promoting factors and by removing ammonia that might be inhibitory to algal growth (Paerl and Kellar, 1978; Mouget *et al*., 1995; Fukami *et al*., 1997; Gonzalez and Bashan, 2000; Källqvist and Svenson, 2003; Ho *et al*., 2006). Besides advantages mentioned above, algae and bacterial relationship might be antagonistic, having negative effects on wastewater treatment process. Microalgal growth is usually slow because of their larger size than the heterotrophic bacteria (Fenchel, 1974). Microalgae might have hazardous (detrimental) effects on bacterial growth by increasing pH (increased up to 10.6 due to algal respiration), Dissolved Oxygen Concentration (DOC), temperature, or by releasing inhibitory extracellular metabolites (Green *et al*., 1996; Oswald, 2003; Schumacher *et al*., 2003; Munoz and Guieysse, 2006). One such example is the release of cyanotoxins by cyanobacteria, which inhibit nitrification (Makarewicz *et al*., 2009). Similarly algal growth may also be inhibited by release of some extracellular metabolites by bacteria Bacterial growth may also inhibit algae by producing algicidal extracellular metabolites (Fukami *et al*., 1997).

Removal of Nutrients/pollutants

Naturally, microbial consortia degrade pollutants more rapidly as compared to a single species (Ramakrishnan *et al*., 2011). Wastewaters from a potato processing industry and from a treated liquid fraction of pig manure inoculated with *Chlorella sorokiniana* and aerobic bacteria, resulted in increased lipid contents of the produced algal biomass (Hernández *et al*., 2012). The efficiency of algal-bacterial associations for the remediation of industrial wastewaters of a pond studied by utilizing several *Chlorella* sp., *Scenedemus* sp., *Stichococcus* strain and *Phormidium* sp. with bacterial strains collected from the pond, showed significant decrease in pollutants: phenols were removed up to 85%, anionic surface active substances up to 73%, oil spills up to 96%, copper up to 62%, nickel up to 62%, zinc up to 90%, manganese up to 70% and iron up to 64% (Safonova *et al*., 2004)

The microalgal (*Scenedesmus obliquus* and *Chlorella vulgaris*) and bacterial (*Raoultellaterrigena* and *Pantoeaagglomerans*) consortia appeared to be promising candidates for the bioremediation of olive wash water (Maza-Márquez *et al*., 2014). A schematic layout of construction of sunlight mediated eco-friendly microalgae-bacteria consortia and its auxillary benefits (Fig. 2).

Carbon dioxide sequestration

Microalgae/cyanobacteria have the ability to fix 10-50 times higher CO_2 than the terrestrial plants. Thus they are considered to be more efficient in capturing/fixing CO_2. For algae cultivation main CO_2 sources being utilized so far are

a) Atmospheric CO_2 (380ppm)
b) CO_2 generated in photobioreactors
c) Flue gas released by coal power plants (Yun et al., 1997; Wang *et al*., 2008).

The need is to exploit algal strains which have higher CO_2 sequestration ability, especially those tolerant to high levels of CO_2 and temperature, their appropriate bacterial partners and construction of suitable bioreactors (Chinnasamy *et al*., 2009; Subashchandrabose *et al*., 2011).

Biodiesel/Biohydrogen production

Hydrogen is amongst the most efficient energy sources. Due to the current energy scenario and environmental concerns, biohydrogen production is need of the day (Bahadar and Khan, 2013; Milledge and Heaven, 2014). Biohydrogen can be produced by anaerobic bacteria or its consortia by dark fermentation process, purple photosynthetic non-sulfur bacteria by photofermentation process and microalgae/cyanobacteria by direct or indirect pyrolysis (Bahadar and Khan, 2013; Cardoso *et al*., 2014; Oliveiria *et al*., 2014). *Chlamydomonas*, *Chlorella* and *Scenedesmus* are the potential hydrogen producers (Milledge and Heaven, 2014).

There is little information available about the biohydrogen production by employing algal-bacterial consortium (Wu *et al*., 2012). Lakatos *et al*. (2014) proposed that bacteria maintained anaerobic environment by consuming O_2 generated by algae, which is suitable for algal hydrogen production. Wirth *et al*. (2015) demonstrated that with the help of mutualistic bacterial flora/partners (AB+S culture) which consumed O_2 being produced by microalgae

(*Chlamydomonas* sp. and *Scenedesmus* sp.), algae produces H_2 without any damage to algal photosynthetic machinery. Additionally algal-bacterial consortia also produce other biofuels; biodiesel and biogas.

Potential of residual microbial biomass as animal/fish feed

Microalgal biomass is a potential source of animal feed/supplement. Some microalgal species contain high amounts of vitamins and/or ω-3PUFAs, which enhance nutritional values of animal meat and the products (Franklin *et al.*, 1999; Thajuddin and Subramanian, 2005; Spolaore *et al*., 2006; Chishti, 2007; Mendes *et al*., 2009). Dried algal residual cakes posse's high contents of proteins, upto 60% of the dry matter in some cases, and thus represent valuable substitute of some conventional sources of proteins such as soybean meal (Becker, 2007). It is to be noticed at this level that some microalgae posses extremely fast growth rates and have the ability of doubling their biomass within 24 hours (Chishti, 2007). *Chlorella* sp., *Scenedesmus* sp*., Dunaliella* sp. and cyanobacteria *Aphanizomenonflos-aquae* and *Spirulina* sp. are being used as sources of chemicals and nutrient-dense foods. They posses significant amounts of certain biomolecules of commercial importance including: lentin, astaxanthin, phycobillins, carotenoids, polyunsaturated fatty acids (PUFAs), chlorophyll, lipids, minerals, pigments and proteins (Yakoob *et al*., 2014). They also may have certain probiotic compounds that enhance health (Kay and Barton, 1991). Microalgal largest current application is its use as feed in aquaculture. Microalgae are being used as live or dried biomass in shrimp, bivalve and fingerlings and fish fry production (Benemann, 1992; Spolaore *et al*., 2006). Many microalgal species have been evaluated for their nutrient and biochemical compounds and their potential as a feed supplement for animals/livestock established (Singh and Gu, 2010). Application of microalgal biomass as animal feed is known since 1950s, but attempts to utilize lipid-extracted/de-fatted microalgae as/in animal feed is a new approach which confers the biodiesel production (Dib *et al*., 2012; Austic *et al*., 2013). Microalgae dual application as an active source of biofuel and animal/fish feed will help alleviate the green house effects associated with current energy and food productions. The de-fatted microalgal biomass obtained after biofuel production may be promising carbon-neutral animal feed supplements (Becker, 1994; Brune *et al*., 2009; Shields and Lapatsch, 2012).

From the above descriptions it becomes quite clear that microalgal de-fatted biomass of different origin in different feeding ratios exert differing effects when fed to different animal species. Moreover, the variations in the feeding of microalgal biomass derived feed from different cultivation sources must be verified. It is expected that future detailed investigations in this regard will identify target oriented feeding regimes.

Prospects from Pakistan

All the important sources such as nutrients, $CO_2$and sunlight, required to establish a microalgal-bacterial consortium, are abundantly available throughout the year in Pakistan. Pakistan lies in the area which is highly suitable for the microalgal cultivation (Fig. 3) whereas Fig. 4 depicts the sunlight availability in the country throughout the year. Manzoor *et al*. (2015) reported potentials of some native oleaginous microalgal species from Pakistan which are capable of producing high oil yields (Table I).

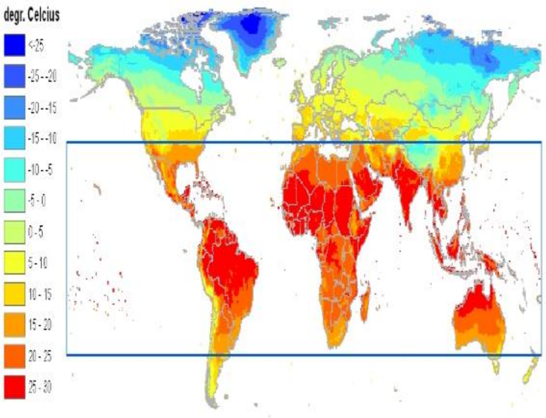
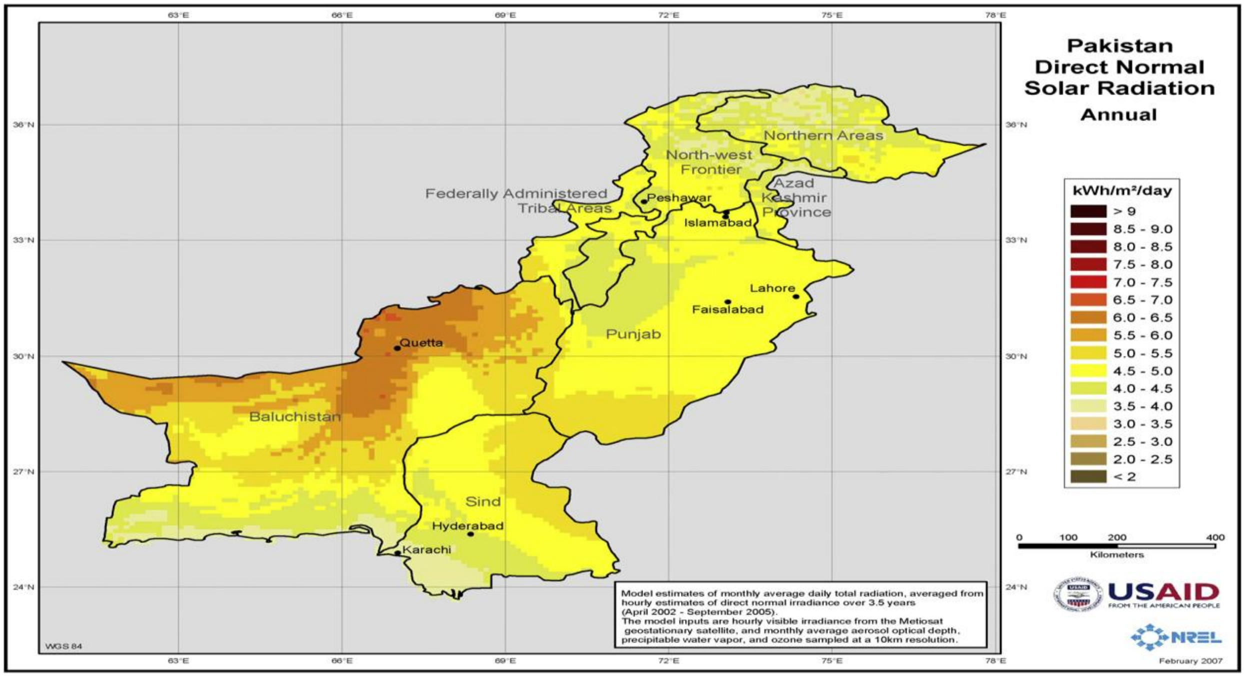


Figure 3: Map showing suitable sites for algal growth (Florentinus *et al*., 2008).

Use of biomass as energy source is not new for mankind. Biomass is an economical option for generating fuel in Pakistan (Mirza *et al*., 2008). Pakistan is characterized with large agricultural and livestock sector and resultantly copious quantities of agriculture and crop residues such as rice husk, wheat straw, cane trash, cotton sticks, bagasse, municipal solid waste, animal residue and poultry litter, etc are produced whose disposal is a big challenge. Availability of waste biomass is also widespread in urban areas of Pakistan where about 1 million tones of animal manure, 55000 tones of solid wastes and 225000 tones of crop residue productions/day are estimated. Waste biomass is abundantly available in many areas of Pakistan. Annual production of different crops and residues being generated is depicted clearly in Table II, adapted from (Bhutto *et al* 2011).



Model estimates of monthly average daily total radiation, averaged from hourly estimates of direct normal irradiance over 3.5 years (April 2002 - September 2005).
The model inputs are hourly visible irradiance from the Metiosat geostationary satellite, and monthly average aerosol optical depth, precipitable water vapor, and ozone sampled at a 10km resolution.

Figure 4: Map showing sunlight distribution in Pakistan developed by USAID and NREL (NREL, 2010)

Annual production of sugarcane in Pakistan is estimated to be 47,244,100 million tons (Ho, 2006). Sugarcane bagasse and molasses are the byproducts of sugar industry (Satyawali and Balakrishnan, 2008). More than 12 million tons of sugar cane bagasse is produced annually in Pakistan (Dawn News, 2012). Lohrey and Kochergin (2012) estimated that 5.8 million litres of biodiesel can be produced annually by a 530 hactre farm while utilizing the wastes available from a 10,000 ton/day sugar cane mill and these results in 15% reduction in CO_2 emissions. Mu *et al.* (2015) analyzed the growth of *Chlorella protothecoides* in sugarcane bagasse hydrolysate and found maximum lipid productivity of 1.19g/L/d and biomass production of 24.01g/L under fed batch culture conditions. Rattanapoltee and Kaewkannetra (2014) investigated sugarcane bagasse as growth medium for *Scenedesmus acutus* and maximum productivity and biomass concentration were obtained at 160.42 mg/L/d and 3.85g/L. There is an increase in biomass production of *Scenedesmus bijugus* and *Chlorella vulgaris* in a study where sugarcane molasses (hydrolyzed) and cassava wastewater were being used as low cost substrates (Vidotti *et al*., 2014).

Conclusion

Microalgae are globally used as feedstock for biofuel/biodiesel production. There is need to exploit solar energy which is available abundantly in Pakistan all over the year, harness indigenous/native oleaginous microalgal strains for biofuels production and wastes treatment concomitantly. Moreover coupling cyanobacteria/microalgae with bacteria increases the efficiency of removal of pollutants and nutrients from the wastewaters. This will help alleviating the problem and crisis of energy which the country is facing today. The above discussed studies allow proposing wastes as economically feasible substrates for the production of biofuels.

Conflict of Interest

There is no conflict of interest.

Table I: Some microalgal species, reported from Pakistan, documented for their oleaginous potential and cultivatibility in wastes.

Sr. No.	Species Reference(s)	oil content % Reference(s)	Cultivation in waste(s) Reference(s)
1.	*Scenedesmus bijuga* (Leghari *et al*., 2001; Korai *et al*., 2008; Aliya *et al*., 2009)	35.24-34.10% (Liu *et al*., 2011)	Mixotrophic growth potential of *S.bijuga*, *Chlamydomonas globosa, Chlorella minutissima* in media supplemented with poultry litter extract and treated/untreated carpet industry wastewaters (Bhatnagar, *et al*., 2011); sewage wastewaters (Ajayan *et al*., 2011).
2.	*Chlorella vulgaris* (Leghari *et al*., 2001; Jahangir *et al*., 2001; Waqar-ul-Haq *et al*., 2008; Korai *et al*., 2008; Ali *et al*., 2010)	14-56% DCW (Gouveia and Oliveira, 2009)	Raw and pre-treated cane sugar mill wastes (Travieso *et al*., 1996) Orange peels extract (Park *et al*., 2014)
3.	*Scenedesmus obliquus* (Aliya *et al.,* 2009; Ali *et al*., 2010)	12-14% DCW (Becker,1994)	Fish pond discharge and poultry litter (Mandal and Mallick, 2012); brewery wastewaters (Mata *et al*., 2013)
4.	*Botrycoccus braunii* (Korai *et al*., 2008)	25-86% DCW (Dayananda *et al*., 2006)	Piggery wastewater (An *et al*., 2003); livestock wastewater (Schenk *et al*., 2008)
5.	*Scenedesmus quadricauda* (Jehangir *et al*., 2001; Korai *et al*., 2008; Aliya *et al*., 2009; Ali *et al*., 2010)	19.9% DCW (Mohapatra, 2006)	Digested wastewater (Xiao *et al*., 2011)
6.	*Chlorella pyrenoidosa* (Nazneen, 1984; Korai *et al*., 2008)	2% DCW (Mata *et.al*., 2010)	Dairy wastewater (DWW) (Kothari *et al*., 2012)

Table 2: Different crops and residues generated annually in Pakistan (Bhutto *et al.,* 2011)

Major Crop	Annual Production	Residue
Sugarcane	49,373	Bagaasse, leaves and tops
Dry chilly	1877	Stalks
Rice	6883	Husks, Straws, Stalks
Wheat	23,864	Pod, Stalks
Cotton	3000	Boll shell, Husk, Stalks
Maize	296	Cobs, Stalks
Barley	82	Stalks
Bajra	470	Cobs, Husks, Stalks

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ISSN 1016-1597 (Print)
ISSN 2313-8556 (online)

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