# Potential of Blue-Green Algae in Wastewater Treatment

**Pushan Bag, Preeti Ansolia, S. K. Mandotra, and Amit K. Bajhaiya**

## 1 Introduction

Being the most primitive life form on earth, ranging from prokaryotes to eukaryotes, algae are a large group of photosynthetic multicellular/unicellular organisms characterized in two classes, macro- and microalgae. Large seaweeds such as green algae, red algae and brown kelp come under macroalgae, whereas microalgae are mainly freshwater-born single-cell organism, known to produce 70% of total atmospheric oxygen. Microalgae can be classified in several groups depending on cellular structure, life cycle and pigmentation. Blue-green algae (BGA) is the most important one among them due to its high growth rate and considered one of the most economic biomass-producing organisms. In general, the term blue green is used for the cyanobacteria (CB), which are group of gram-negative photosynthetic bacteria that have colonized earth over 3.5 billion years ago. Though they are bacteria, they have several features common with algae, and they can be naturally found in a wide variety of environments including river, ponds, lakes and streams. CB are considered as the predecessors of modern-day chloroplast. They are also known to possess great deal of morphological and metabolic diversity, which makes them

P. Bag
Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, Umea, Sweden

P. Ansolia
Department of Pharmacy, Radharaman Institute of Technology & Science (RITS), Bhopal, India

S. K. Mandotra
Department of Botany, Punjab University, Chandigarh, Punjab, India

A. K. Bajhaiya (✉)
Department of Chemistry, Chemical Biological Centre (KBC), Umeå University, Umea, Sweden
e-mail: amit.bajhaiya@umu.se



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extraordinary repertoire of different chemical compounds with application in food, feed, cosmetic, nutritional, pharmaceutical and even in biofuel industry. Although the BGA utilization is more than centuries old (*Nostoc* in Asia and *Spirulina* in Africa and Mexico), the purposeful cultivation of BGA has started only few decades ago. Apart from its increasing uses in agriculture, food and cosmetic industry, the emerging trend is to use BGA for wastewater treatment. In this chapter we will mainly focus on prospects of BGA in wastewater and its pros and cons.

## 2 Potential Applications of BGA

BGA has gained immense attentions due to its multivariant usage in biotechnology, specifically in agricultural biotechnology, natural products, cosmetics industry and production of numerous secondary metabolites including vitamins, enzymes and pharmaceuticals and most recently in wastewater treatment since the basic idea was given by Caldwell in the early 1940s.

### *2.1 Agricultural Biotechnology*

Ability of BGA to photosynthesize and fix atmospheric $N_2$ gives inherent fertility to soil and explains how rice has be cultivated without any external supplies of $N_2$ even before invention of fertilizer. Field trails shows that N contribution by BGA is 20–30 kg/ha (Goyal 1993); thus farmers can get 20–30 kg/ha nitrogen without using any N supplements. Application of compost or dry algal mass in soil is more effective due to the availability of secondary N product in the field water. Effects have also been seen in other crop plants such as barley, oat, tomato, sugarcane, etc. BGA also helps in sustaining crop yield due to its multivalent capacity to produce vitamins, carbohydrates and growth hormones (Mishra et al. 1989; Kaushik 1998). Such N fixing cyanobacteria are listed in Table 1.

Apart from N fixation, BGA can be used as potential organisms to reclaim salinity-affected soils. *Anabaena torulosa* have been found to grow and enrich N status of saline coastal soils. Most of the sodium removed by BGA remains extracellularly trapped in their mucopolysaccharide sheaths (Apte and Thomas 1997); therefore, permanent salt removal from saline soils may not be possible, since $Na^+$ is released back into the soil subsequent to the death and decay of cyanobacteria. However, 25–38% of sodium can be removed by the removal of top soil.

Another major nutrient for plant growth is phosphate, which is very limiting in natural ecosystem, even if added externally; it is immediately converted in insoluble phosphate compounds which cannot be taken up by plants. BGA has the capability to solubilize phosphate compounds such as $(Ca)_3(PO_4)_2$ (tricalcium phosphate), $FePO_4$ (ferric orthophosphate), $AlPO_4$ (aluminium phosphate) and

**Table 1** Nitrogen-fixing cyanobacterial species

| Unicellular | Filamentous: Heterocystous | Filamentous: Non-heterocystous | References |
|---|---|---|---|
| *Aphanothece, Chroococcidiopsis, Dermocarpa, Gloeocapsa, Myxosarcina, Pleurocapsa, Synechococcus, Xenococcus* | *Anabaena, Anabaenopsis, Aulosira, Calothrix, Camptylonema, Chlorogloea, Chlorogloeopsis, Cylindrospermum, Fischerella, Gloeotrichia, Haplosiphon, Mastigocladus, Nodularia, Nostoc, Nostochopsis, Rivularia, Scytonema, Scytonematopsis, Stigonema, Tolypothrix, Westiella, Westiellopsis* | *Lyngbya, Microcoleus chthonoplastes, Myxosarcina, Oscillatoria, Plectonema boryanum, Pseudoanabaena, Schizothrix, Trichodesmium* | Vaishampayan et al. (2001), Pereira et al. (2009), Rana et al. (2012), Prasanna et al. (2013) |

Mainly two types of cyanobacteria are mentioned here, i.e., unicellular and filamentous, which can be Heterocystous forming or non-Heterocystous forming

**Table 2** Several agrochemical degrading cyanobacterial species which can be beneficial in wastewater treatment system to degrade mainly toxic pesticides used in cultivation

| Cyanobacterial species | Pesticide degraded | Reference |
|---|---|---|
| *Anabaena* sp., *Microcystis novacekii, Nostoc linckia, N. muscorum, Oscillatoria animalis, Phormidium foveolarum.* | Methyl parathion | Fioravante et al. (2010) |
| *Anabaena fertilissima, Nostoc muscorum* | Monocrotophos, malathion, dichlorovos , phosphomidon | Subramanian et al. (1994) |
| *Anabaena* sp. *A. azotica, A. cylindrica, Cyanothece* sp., *Nodularia* sp., *Nostoc* sp., *Oscillatoria* sp., *Synechococcus* sp. | Lindane | El-Bestawy et al. (2007); Zhang et al. (2012) |
| *Synechocystis* sp. Strain PUPCCC 64 | Anilofos | Singh et al. (2013) |
| *Synechocystis* sp. Strain PUPCCC 64 | Chlorpyrifos | Singh et al. (2011) |

Though specificity unknown but found to have significant effect under laboratory conditions

$Ca_5(PO_4)_3(OH)$ (hydroxylapatite) (Vaishampayan et al. 2001). Cyanobacteria such as *Anabaena doliolum*, *A. torulosa*, *Nostoc carneum* and *N. Piscinale* decompose and mineralize phosphate into soluble organic phosphates/orthophosphates and can also mobilize inorganic phosphates by means of extracellular phosphatases (Prasanna et al. 2013). Intensive dependence on agrochemicals has also brought significant pollution in soil ecosystems, and recent reports also suggest that cyanobacteria are capable to degrade agrochemicals to a certain extent (Subashchandrabose et al. 2013). Several agrochemicals degrading BGA have been listed in Table 2. Recent studies have shown that cyanobacteria are helpful in producing phytohormones, such as *Anabaena*, *Anabaenopsis*, *Calothrix*, *Chlorogloeopsis*, *Chroococcidiopsis*, *Cylindrospermum*, *Gloeothece*, *Nostoc*, *Oscillatoria*, *Plectonema*, *Phormidium* and *Synechocystis*, and help in production of auxins, whereas *Anabaena*, *Calothrix*, *Chlorogloeopsis*, *Chroococcidiopsis* and *Rhodospirillum* produce cytokines (Singh et al. 2016).

## *2.2 Food*

BGA, specifically *Spirulina*, contains highest amount of proteins around 65% followed by soybean, dried milk (35%) and animal and fish flesh (15–25%). History of eating *Spirulina* by North African people and *Nostoc commune* by Chinese people goes back to 317–420 AD during the rule of Jin Dynasty. Apart from being rich in protein, *Spirulina* also contains significant amount of vitamins, lipids and other health-promoting substances, which make them commercially produced and sold in the names of Zyrulina, Recolina, etc. Dry powder containing capsules of *Aphanizomenon flos-aquae*, under the trade name of Klamath's Best® Blue Green Algae by Klamath Valley Botanicals LLC, USA, is famous in the USA, Germany, Canada, Korea, Japan and Austria due to having up to 20 antioxidants, 68 minerals and 70 trace elements, all amino acids, B vitamins and other important enzymes (Chakdar et al. 2012).

## *2.3 Natural Colours*

Having the word 'green' in the names, BGA produces huge amount phycobilin and carotenoids apart from chlorophyll, which comprises up to 60% of total soluble proteins (Bogorad 1975). Phycocyanin (PC), phycoerythrin (PE) and long-chain terpenoids are among other phycobilins that have gained popularity as natural colourant for having nontoxic and environmental friendly effects. They are produced commercially from *Spirulina platensis* and *Anabaena*; several companies have incorporated them in their products containing natural colourant such as Dainippon Ink and Chemicals (Sakura, Japan) which developed a product called 'Lina blue' (PC extract from *S. platensis*), which is used in chewing gum, ice sherbets, popsicles, candies, soft drinks, dairy products and wasabi. High molar absorbance coefficients, high fluorescence quantum yield, large Stokes shift, high oligomer stability and high photostability properties make phycobiliproteins very powerful and highly sensitive fluorescent reagents. Purified native phycobiliproteins and their subunits fluoresce strongly; they have been widely used as external labels for cell sorting and analysis and a wide range of other fluorescence-based assays (Tooley et al. 2001).

### 2.4 Cosmetic Industry

BGA, namely, *Spirulina*, has gained major market uprising in cosmetic industry due to its natural colouring properties. Properties such as repairing signs of early skin aging, tightening effect, preventing stretch mark formation, improving moisturizing balance of skin, increasing skin's immunity naturally, lightning skin complexion and removing dead skin cells and photoprotective effect without having side effects have given *Spirulina* an edge over other synthetic products.

### 2.5 Bioactive Molecules and Antibiotics

The usage of BGA as medicine has long been established since 1500 BC, and compounds from *Anabaena*, *Nostoc* and *Oscillatoria* are known to produce an array of bioactive secondary metabolites, some of which are shown to have antibacterial and antifungal properties. A diterpenoid from *N. commune*, noscomin, showed antibacterial activity against *Bacillus cereus*, *Staphylococcus epidermidis* and *Escherichia coli* (Jaki et al. 1999). Natural products of *Nostoc* sp. are effective against *Cryptococcus* sp. as a causal agent of secondary fungal infections in patients with AIDS (Kuwaki et al. 2002). Anticancer properties have also been identified in *Scytonema* sp., *Phormidium tenue* and *Anabaena variables*. Cryptophycin-1, isolated from a *Nostoc* sp., has been found to have cytotoxic activity against nasopharyngeal carcinoma and human colorectal adenocarcinoma cell lines (Trimurtulu et al. 1994). Several modified bioactive compounds with reduced level of toxicity from *Spirulina* are also in second clinical trial phases as well (Tan 2010).

### 2.6 Biofuels

Cyanobacteria can be used for energy production, such as through production of hydrogen. Advantage of using natural energy produced by algae is its eco-friendly nature and almost no side effect or production of any pollutant (Dutta et al. 2005). Cyanobacteria mainly produces hydrogen as a secondary product of nitrogen fixation or by reversible activity of hydrogenase enzyme. More than 14 cyanobacterial genera including *Anabaena*, *Calothrix*, *Oscillatoria*, *Cyanothece*, *Nostoc*, *Synechococcus*, *Microcystis*, *Gloeobacter*, *Aphanocapsa*, *Chroococcidiopsis* and *Microcoleus* are known for their ability to produce hydrogen gas under various culture conditions. Several hydrogen producing BGA are mentioned in Table 3. Recently large-scale production of hydrogen in several bioreactors has been tried successfully and almost on its way to commercialisation (Dutta et al. 2005).

**Table 3** Several hydrogen-producing blue green algae

| Species of cyanobacteria | Growth conditions | Maximum hydrogen production | Reference |
|---|---|---|---|
| *Anabaena sp. PCC 7120* | Air, 20 μE $m^{-2}$ $s^{-1}$ | 2.6 μmol $mg^{-1}$ chl a $h^{-1}$ | Masukawa et al. (2002) |
| *Anabaena cylindrical lAMM-l* | Air, 20 μE $m^{-2}$ $s^{-1}$ | 2.1 μmol $mg^{-1}$ chl a $h^{-1}$ | Masukawa et al. (2002) |
| *Anabaena variabilis AVMl3* | Air and 1% $CO_2$, 100 μE $m^{-2}$ $s^{-1}$ | 68 μmol $mg^{-1}$ chl a $h^{-1}$ | Happe et al. (2000) |
| *Anabaena variabilis PK84* | Air and 2 % $CO_2$, 113 μE $m^{-2}$ $s^{-1}$ | 32.3 μmol $mg^{-1}$ chl a $h^{-1}$ | Tsygankov et al. (1999) |
| *Anabaena variabilis ATCC 29413* | 73% Air, 25% $N_2$, 2 % $CO_2$, 90 μE $m^{-2}$ $s^{-1}$ | 46.16 μmol $mg^{-1}$ chl a $h^{-1}$ | Sveshnikov et al. (1997) |
| *Aphanocapsa montana* | Air, photon fluence rate 290 μE $m^{-2}$ $s^{-1}$ | 0.40 μmol $mg^{-1}$ chl a $h^{-1}$ | Howarth and Codd (1985) |
| *Chroococcidiopsis thermalis* | Ar and 1% $CO_2$ | 0.7 μmol $mg^{-1}$ chl a $h^{-1}$ | Serebryakova et al. (2000) |
| *Gloeocapsa alpicola CALU 743* | Sulfur free 4% $CO_2$; 25 μmol photons $m^{-2}$ $s^{-1}$ | 0.58 μmol $mg^{-1}$ protein | Antal and Lindblad (2005) |
| *Gloeobacter PCC 7421* | Air, photon fluence rate 20 μE $m^{-2}$ $s^{-1}$ | 1.38 μmol $mg^{-1}$ chl a $h^{-1}$ | Moezelaar et al. (1996) |
| *Microcystis PCC 7820* | Air, photon fluence rate 20 μE $m^{-2}$ $s^{-1}$ | 0.16 μmol $mg^{-1}$ chl a $h^{-1}$ | Moezelaar et al. (1996) |
| *Nostoc commune lAMM-l 3* | Air, 20 μE $m^{-2}$ $s^{-1}$ | 0.25 μmol $mg^{-1}$ chl a $h^{-1}$ | Masukawa et al. (2002) |
| *Synechococcus PCC 6803* | Air, photon fluence rate 20 μE $m^{-2}$ $s^{-1}$ | 0.26 μmol $mg^{-1}$ chl a $h^{-1}$ | Moezelaar et al. (1996) |
| *Synechococcus PCC 6301* | Air, photon fluence rate 20 μE $m^{-2}$ $s^{-1}$ | 0.09 μmol $mg^{-1}$ chl a $h^{-1}$ | Howarth and Codd (1985) |
| *Synechococcus PCC 6308* | Air, photon fluence rate 20 μE $m^{-2}$ $s^{-1}$ | 0.13 μmol $mg^{-1}$ chl a $h^{-1}$ | Howarth and Codd (1985) |
| *Synechococcus PCC 6714* | Air, photon fluence rate 20 μE $m^{-2}$ $s^{-1}$ | 0.07 μmol $mg^{-1}$ chl a $h^{-1}$ | Howarth and Codd (1985) |

Mainly tested in laboratory conditions and found to have significant hydrogen production rate. Mostly these species are from Anabaena and Synechococcus

## 2.7 *Wastewater Treatment*

The usage of BGA in wastewater treatment is the most recent activities of cyanobacteria, which are discussed below. Mostly BGA is used in combination with traditional wastewater treatment process.

### Wastewater Composition and Related Hazards

Wastewater is a by-product of domestic, industrial, agricultural and commercial waste. By definition wastewater is ‘used water from any combination of domestic, industrial, commercial or agricultural activities, surface runoff or stormwater and any sewer inflow or sewer infiltration' (Winfrey and Tilley 2016). Depending on the categories of sources, wastewater compositions are broadly classified into three categories which can come from all of the above-mentioned sources.

### Chemical Compositions

Depending on sources, wastewater can contain a wide range of chemicals. Most harmful chemicals mainly come from industrial and commercial wastes which mainly contain heavy metals, including mercury, lead and chromium along with paints and other ammonium compounds from cosmetic industries. Some agricultural wastes such as urea, drugs, hormones, pesticides, fertilizers and primary and secondary nitrogenous and sulphur compounds are also there. On the other hand, faeces, hairs, food, vomit, paper fibres, plant material, humus, etc. come from domestic chemical wastes. Domestic wastewater is classified into two different classes such as grey water and black water. Grey water is all wastewater that is generated in household or office building sources without faecal contamination. Therefore, by definition, grey water does not include the discharge of toilets or highly faecally contaminated wastewater, which is designated sewage or black water and contains human waste.

Most of the times, chemical contaminates are from nitrogenous compounds, mainly nitrates. Main problems are related to conversion of nitrate to nitrite in the digestive system, which can cause severe problems due to its high absorption rate in the blood stream, where it binds to haemoglobin and forms methaemoglobin and eventually blocks the binding of oxygen creating an oxygen scarcity in blood.

### Biological Compositions

Biological and chemical compositions of wastewater are very much well connected since most of the domestic wastewater contains biologically active organisms from human body or materials used in human households. There are mainly four types of major biological components of wastewater, which almost contains all possible disease-causing microorganisms, such as virus (hepatitis, rotavirus), bacteria (*Salmonella*, *Shigella*, *Campylobacter*, *Vibrio cholerae*) and protozoa (*Entamoeba*, *Giardia*). Apart from this, wastewater may also contain parasites such as Helminths (Ascaris).

There are several methods of wastewater treatment; most used and accepted on are the conventional processes by using disinfectant or by primary/secondary/tertiary treatment processes. But recent developments of industrial chemistry and ecological studies have also showed some emerging promises in natural ways of wastewater treatments.

## Conventional Processes

### Disinfecting Agents

The process of disinfection usually involves the injection of a solution of chlorine at the head end of a chlorine contact basin. The dosage of chlorine depends on the strength of the wastewater and other factors; however the dosages of 5–15 mg/l are commonly used. The ultraviolet (UV) and ozone irradiations can also be used for disinfecting wastewater; however these methods of disinfection are not in common use. The chlorine contact basins are mostly designed as rectangular channels, with obstructs to prevent short-circuiting and to give a contact time of about 30 minutes. However, in some specific conditions or to meet the advanced wastewater treatment requirements, a chlorine contact time can be increased to as long as 120 minutes so that it can meet the requirement for specific irrigation uses. In general, the bactericidal effects of chlorine and other commonly used disinfectants are dependent on the pH, organic content, contact time and effluent temperature (Singh 2017).

### Preliminary Treatment

The idea of preliminary treatment is to remove large solid materials such as wood, pieces of glass, papers, plastic sand, etc. It helps to remove any floating or sedimented material and reduces the overall volume of the liquid. Pretreatment mainly includes a grit removal chamber where the flow of the liquid is controlled carefully to settle down the stones, sands and other solid materials from the liquid, but remaining suspended organic and inorganic material remains in the water. For this there are several screening processes such as coarse screening, fine screening, shredding, flow measuring, pumping and pre-aeration for further downstream process. Sometimes some disinfecting agents are also used to remove odour and to improve settling of grids (Fig. 1). Main goal of coarse screening is to remove materials that can damage the instrument; on the other hand, most of the fine screening is done to remove material that can block channels and other tubes in the machine; these also sometime couple with sedimentation process in the primary treatment.

Shredding is a culmination process where wastewater is prepared for sludge treatment. Culminators are loaded in a channel, and wastewater is passed through it where the blade of the culminator cuts down the rags until they can pass through the openings. Some advanced treatment plants also have specific shaped openings for more controlled shredding. After shredding is it send to a grit removal tank with a motive of removing inert material with a specific gravity of 2.65. Grit removal chambers are designed with specific size-dependent removal such as 0.011 inch with 65 meshes or sometimes 0.007 inches for activated sludge treatment. Most of the times, grit removal chamber is connected to an external sewer system and sanitary system to store excessive grit materials. There are several grit removing chambers such as (a) horizontal grit chamber, (b) detritus tanks, (c) aerated grit chambers, etc. After successful grit removal, raw sewage pumps take the liquid further to the primary treatment chamber and to sludge treatment chamber.

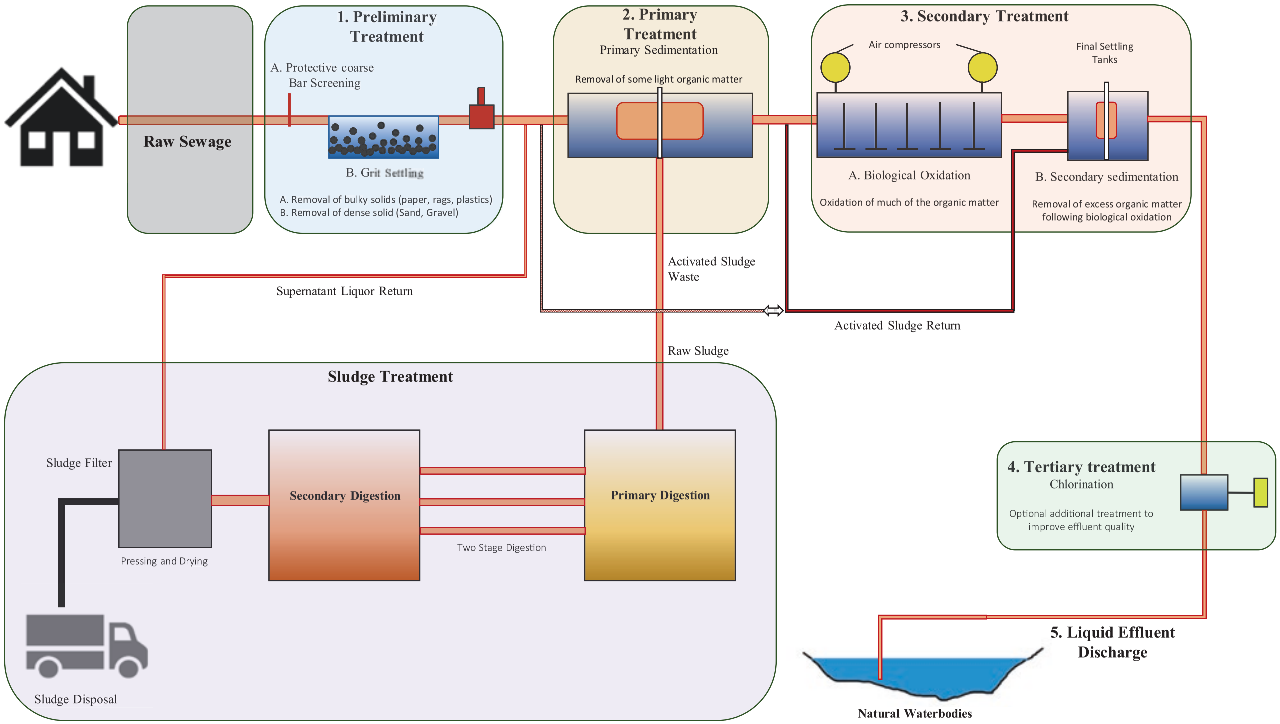


**Fig. 1** Flow diagram of wastewater treatment in a typical plant, starting from raw sewage to sludge treatment and sludge disposal. First raw sludge is passed through preliminary treatment chamber where bulky and dense solids are removed, followed by treatment through primary treatment chamber to remove organic matter. Then, in the secondary treatment chamber, residual sludge is biologically oxidized and allowed for sedimentation. On the final step before releasing the liquid effluent to natural water bodies, it is treated with chlorine to improve the quality of the effluent. All residuals from primary and secondary sedimentation are released into sludge treatment chambers where it is passed through two digestion chambers and finally dried and disposed. Dense and bulky solids from preliminary treatment are directly moved for drying chamber in sludge treatment chambers, but sometimes depending on the type of sludge, it may be passed through sludge digestion chambers as well

Sometimes several additional preparation processes are added before sending it to primary treatment. This is mainly to improve wastewater treatability, providing grease separation, odour control and flocculation. Some pretreatment is also conjugated with primary treatment in case of domestic grey water treatments.

### Primary Treatment

Primary treatment is mainly separating dissolved/colloidal organic and inorganic material by mainly filtration, sedimentation, phase separation or flotation. Previously primary treatment was considered only for domestic wastewater treatment. Colloidal suspensions of fine metals and organic materials are mainly removed by filtration through filters having pore size less than the particles. Particles of size more than colloids are mainly removed by gravity separation. Nonpolar organic substances are also separated by sedimentation. Containers like the API oil-water separator are specifically designed to separate nonpolar liquids (Weber 2004). Phase separation is mainly used to remove oils and grease by passing through a nonaqueous phase. Sometimes oils are saponified and then phase separated. Sometimes ion exchange and reverse phase osmosis are also used to separate nonpolar substances. The effluent from primary treatment is known as primary effluent.

Though primary treatment typically does not involve any chemical treatment, recently it has been observed that the plants use chemicals to coagulate colloidal materials (Grandclément et al. 2017). Depending on this primary treatment is classified into two types.

(a) Plain sedimentation is removal of heavy materials by gravitational field followed by clearing the bottom of the basin. Furthermore, several skimming devices are also installed here to remove the floatable substances such as scums, oil, grease, etc. which is further connected to sludge chamber. Successful removal from domestic water can comprise up to 40% of total BOD and 70% total suspended solids. The most important parameter for this is flow rate, which is very difficult to maintain due to contentious sedimentation of waste in the basin. Recent developments of high rate settlers provide better results due to addition of several trays and tubes in the basin for better settlement and maintaining a proper flow rate. But the problem of slime growth is never possible to remove fully.

(b) Sedimentation with chemical coagulant is introduced due to high amount of phosphorus waste in the industrial wastewater. These chemical coagulants are not at all used in domestic wastewater due to economic issues, but they are very effective to bring down the BOD for further treatments. Chemicals that are used singularly or in combination are salts of iron or aluminium, lime and synthetic organic polyelectrolytes (Yu et al. 2017).

(c) There are also some other methods such as extensive aeration or involving ponds or sometime with no primary treatment at all.

### Secondary Treatment

Motive of secondary treatment is to remove soluble organic and inorganic substances mainly by chemical and biological-chemical oxidation; it may help in removing persistent organic and inorganic material mainly sulphur and phosphorus compounds. Sometimes chemical oxidation is carried out by adding ozone or chlorine to remove biological contaminants (virus and bacteria). Chemical oxidation is widely used for disinfection. Biological oxidation is mainly used for agricultural wastes and in sewage treatments mainly by using various microorganisms under controlled environments. Several aerobic microorganisms are used to breakdown organic materials and some inorganic compounds by means of anoxygenic photosynthesis. Most of the times, high rate biological oxidation is done in a very low volume under well-controlled environment which helps microorganisms to grow. In case of biological oxidation of organic material, it is necessary to remove microorganisms from wastewater by sedimentation to get secondary effluent. This sediment tank performs just like the primary treatment chamber. Followed by secondary sedimentation of microorganisms, this biologically degraded waste is known as biological sludge. The common high rate processes involve activated sludge treatment, biofilter or trickling filter, rotating biological contactors (RBC) or ditch filters. Mostly in case of municipal waste, activated sludge treatment is employed in combination with trickling filters to improve BOD.

### Tertiary Treatment

Tertiary and/or advanced wastewater treatment is employed when specific wastewater constituents which cannot be removed by secondary treatment. For the tertiary treatment, individual treatment processes are necessary to remove phosphorus, nitrogen, additional heavy metals, suspended solids, dissolved solids and refractory organic waste. This advanced treatment is usually follows a high-rated secondary treatment and therefore sometimes called as tertiary treatment. However, the advanced treatment processes can be sometime combined with primary or secondary treatment (e.g. chemical addition to primary clarifiers or aeration basins to remove phosphorus) or used in place of secondary treatment (e.g. overland flow treatment of primary effluent). A flow diagram of stepwise treatment of wastewater is shown in Fig. 1.

## Other Treatment Processes

Other treatment processes mainly include low-cost natural processes such as wastewater treatment ponds (which includes anaerobic ponds, facultative ponds, maturation ponds, etc.), overland treatment of wastewater, macrophyte treatment, nutrient film technique, etc.

# 3 Role of BGA in Wastewater Treatment

Recent developments in biotechnology and genetic engineering have opened a new way of treating wastewater with genetically modified microorganisms specifically blue-green algae. Role of BGA in human welfare is quite ancient as the earliest report available is almost 800 BC, but the use in wastewater treatment is very recent as the idea was proposed in 1945 (Caldwell 1946) and experimentally proved in 1957 (Oswald and Gotaas 1957).

The use of BGA in wastewater treatment has increased due to several reasons mentioned below.

1. Doesn't require nutrient-rich medium to grow; only enough amount of water is sufficient.
2. Since they are photosynthetic, so they can increase oxygen levels in water and also can utilize several organic and inorganic materials as a source of anoxygenic photosynthesis.
3. Cyanobacterial biomass is very easy to use in food and feed stock industry.
4. BGA do not produce any toxic substance rather can outperform the growth of other microorganisms

## *3.1 Water Quality Control*

Water quality control can be monitored by monitoring cyanobacterial blooms due to eutrophication in water bodies. There are several strategies for monitoring cyanobacterial content in wastewater; among them the most used one is monitoring cyanobacterial pigment phycocyanin by spectroscopic methods from drones or spectroscope equipped air shuttles (Fig. 2) (Teta et al. 2017). BGA can also have several effects on human life due to its health related hazards coming from several sources mentioned in details below.

Drinking water: Due to the toxins and pigments produced by cyanobacteria, it can be harmful to be present in a certain amount in drinking water. Sometimes boiling water contaminated with high number of BGA may lead to production of more harmful chemicals and may lead to death.

Skin contact: Skin contact with BGA may lead to some irritation, rashes or maybe redness of eye or swelling of lips, etc., due to toxins present in cyanobacteria. Sometimes prolonged exposure to cyanobacteria may also lead to skin tumour formation.

Eating fishes infected with cyanobacteria: Eating fish or other marine seafoods infected with cyanobacteria may be harmful for our body due to toxins such as cylindrospermopsin; it has been identified in the Queensland freshwaters edible flesh of crayfish. Toxins such as 'Paralytic Shellfish Poisoning' (PSP) from the species of blue-green algae have highlighted concerns about possible neurotoxin bioaccumulation in edible mussels and other shellfish.

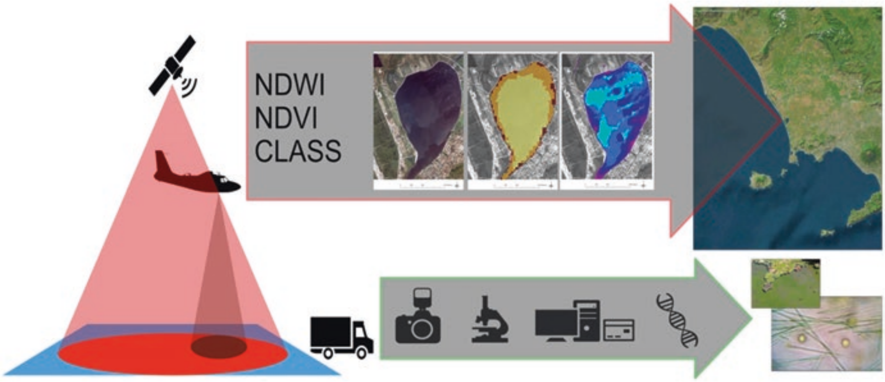


**Fig. 2** Monitoring strategy based on a hierarchical approach, combining remote/proximal and in situ analytical/biotechnological data (https://doi.org/10.1088/1748-9326/aa5649)

But using proper detection and profiling techniques, it can be controlled and monitored for water quality, and this is actually very useful for fisheries and other agricultural fields.

### *3.2 Removal of Inorganic and Organic Toxins*

Several recent studies have shown successful in removal of phosphorus and nitrogenous compounds from nutrient-rich wastewaters (Oswald et al. 1978; Chan et al. 1979). Biological processes such as suspended cultivation are very effective against nitrogenous compound removal; several species such as *Oscillatoria*, *Phormidium*, *Aphanocapsa* and *Westiellopsis* have been found to take up phosphorus and nitrogenous compounds very efficiently from the effluents which in turn reduce the pollution loads of environment (Vijayakumar 2012). There are several limitations of using suspending microalgae, such as perfect operating conditions that are hard to maintain, and it's difficult to maintain monospecificates. Secondly, there are not many effective processes available to separate microalgae from effluents before they can be discharged in the environment. Because of this sole reason, very limited number of stabilization ponds and high rate algal ponds are in use now.

Several immobilization processes such as entrapment of cyanobacteria in matrix (such as agarose, carrageenan, chitson, alginate and polyurethane foam) are used for microalgae immobilization. Process involving immobilized cells has been attempted for the treatment of effluents containing phenols, rubber press wastes, distillery waters, olive oil mill wastes, paper mill sludge, diary wastewaters and textile dye effluents. But these entrapment methods are found not to be very effective in terms of its activity to degrade discharges containing high amount of organic and inorganic compounds.

### 3.3 Maintaining Oxygen Levels in Water

Since BGA mainly uses oxygen to catalyse reactions to remove organic and inorganic wastes, so it is also a very good indicator of dissolved oxygen and hence helps in maintaining proper BOD and COD. Being photosynthetic organisms, they also tend to perform oxygenic photosynthesis and produce a lot dissolved oxygen.

### 3.4 Heavy Metal Treatments

In current situations of mining and mineral processing industries where excessive amount of metals and chemical are used for extra-metallurgical operations are raising concerns as it results in production and discharge of large amount of aqueous effluents with high metal contents which has a drastic effect on nearby water bodies (Vijayakumar 2012). The main concern of this era is to remove these toxic metallic compounds from effluents to an acceptable limit by using cost-effective and environment-friendly processes. Tiny cyanobacteria have really high metal absorption capacity with high doubling rate. These characters of cyanobacteria have encouraged their biomass usages in the detoxification of effluents. Moreover, as cyanobacteria are photosynthetic organisms, so it's more effective in removing heavy metals and detoxification of effluents. The interior pH of cyanobacterial cell is higher (approximately by two units) than the surrounding liquid, so it resists product transfer from the biofilm. Recent studies have revealed that cyanobacteria immobilized in matrix are more potent to remove heavy metals than free-living cyanobacteria. Some of the examples are:

1. An increased uptake of Cu and Fe by 45% and 23% seen in immobilized *Anabaena* compared to free counterpart
2. Another *Anabaena* species *A. Doliolum* showed 15–20% and 10–30 lower Cr and Ni removal by free-living cells when compared to that of immobilized cells.

Mechanism of metal removal is complex process which mainly occurs in two distinct phases: In phase I cations (positively charged ions) bind to negatively charged groups of the cell wall of cyanobacteria very rapidly which makes a negatively charged masking of the cell wall. This promotes the second phase, where the metal ions are taken up depending on the metabolic requirements and conditions of the cells (Pabbi, 2015). One intriguing fact was also proposed that this high metal uptaking property of immobilized cells over the free-living cells actually increases the photosynthetic energy productivity. This is maybe due to high amount cation pumping in the immobilized cells, which creates an H+ imbalance in the cell. This imbalance also increases H+ levels in chloroplast and mitochondria which is then used for ATP production while pumping out the protons to maintain the homeostasis. However, high metal uptaking property of the immobilized cells can also be due to increased permeability of cell wall (Khummongkol et al. 1982). It is also very much possible that immobilized cells have higher degree of successive collision with the metal ions than the free-living cells, which is also one of the major reasons of immobilization.

Mainly heavy metals like mercury, cadmium and lead pose the biggest hazard to human health, in addition to As, Be and Cr which are reported to be carcinogenic. These metals can cause serious damage to aquatic life due to accumulation through the trophic chain, production of toxic effects and teratogenic changes in plants, animals and human beings. This is also because of the remains of heavy metals in the sediments and release in freshwater or mixing of freshwater with heavy metal-contaminated wastewater (Wilde and Benemann 1993).

Traditional methods such as ion exchange, electrochemical treatment, precipitation, evaporation, reverse osmosis and sorption for heavy metal removal from waste streams are costly and not very much effective. Hence, biological approaches have emerged as an alternative remediation for heavy metal contamination. Since the last two decades, extensive study of microorganisms in bioremediation of heavy metals has shown a way to use BGA as the most effective remediating agents.

Most successful heavy metal remediation is dependent heavily on environmental conditions and mostly dependent on pH and temperature. Certain algae such as *Chlorella*, *Scenedesmus*, and *Hydrodictyon* can remove up to 90% of heavy metals from wastewater. Recent studies have found *Phormidium ambiguum* (*Cyanobacterium*), *Pseudochlorococcum typicum* and *Scenedesmus quadricauda var quadrispina* (Chlorophyta) to have high capacity of removing mercury and cadmium (Shanab et al. 2012). Another study has shown biosorption of different toxic heavy metals such as Pb, Cd, Co, Ni, Zn and Cu by exopolysaccharide (EPS) produced by *Paenibacillus jamilae* (Pérez et al. 2008). Another study showed rhizobia has significant roles of extracellular polysaccharides and biofilm formation (Nocelli et al. 2016). A study on *Spirulina platensis* showed significant uptake of chromium ($Cr^{3+}$) in free form rather than in an embayed form (Shashirekha et al. 2008).

These studies including other bioremediating properties give emerging promises for usage of BGA in traditional wastewater treatment plant to reduce cost-effectivity and better results, and large-scale experiments has also been started in the USA and Canada.

## *3.5 Coliform Removal*

Ecotechnologies such as algal- and duckweed-based pond systems are becoming popular for wastewater treatments due to easy and cost-effective removal of pathogens in warm climatic conditions, though the mechanisms are not well understood. Several strategies based of basic physiological conditions are being used to remove coliforms by means of overproduction of BGA, such as increasing temperature (Brissaud et al. 2003; El-Shafai et al. 2007), nutrient deprivation (Van der Steen et al. 2000), sunlight, pH, dissolved oxygen (Davis-Colley et al. 2000) or algal toxins (Oudra et al. 2000).

**Table 4** Comparison of roles of different blue green algae in duckweed and algal ponds in removing coliforms in different seasons during the year

| Location | Season/temp. (°C) | DK | AL | CS | Reference |
|---|---|---|---|---|---|
| | | Removal (log units) | | | |
| Accra, Ghanaa | Wet 24–29 | 3,8 | 4,8 | 4,3 | Ansa (2013) |
| | Dry 30–33 | 3,5 | 4,6 | 4,3 | |
| | Year-round | 3,7 | 4,7 | 4,3 | |
| Kumasi, Ghana | Year-round 24–27 | 4,0 | 5,0 | | Awuah et al. (2004) |
| West Bank, Palestine | Winter 7–13 | 1,0 | 3,1 | | Al-Sa'ed (2000) |
| | Summer 21–27 | 2,0 | 2,7 | | |
| Negev, Isreal | Winter 15–18 | | 2,6 | 2,2 | Van der Steen et al. (2000) |
| | Spring 18–27 | | 2,7 | 2,3 | |
| Belo Horizonte, Brazil | Yearround 20 | | | 6,7 | Von Sperling and Mascarenhas (2005) |

In nutrient-deprived conditions, algal growth not hampered very much due to their capability of photosynthesis, but on the other hand, coliforms lacking nutrient are easily outperformed. While increasing temperature, pH and less dissolved oxygen trigger the algal cultures to produce several super oxides and other free radicals which eventually kill possible coliforms. Again, selective growth of toxin producing algal stains also an effective idea of outperforming the growth of coliforms mainly in maturation ponds.

In both algal and duckweed ponds, faecal coliform levels have been found to be decreasing in different rates due to summer and winter conditions and availability of sunlight as well. Role of algae in duckweed and algal ponds in removing coliforms is represented in Table 4.

## 4 Concluding Remarks

Cyanobacteria being one of the primitive organisms are very simple for genetic engineering and have vast potential in environmental remediation. Several uses of BGA are mentioned here and furthermore are under research. Not only in remediation but also in energy production and usage in daily life make BGA more commercially valuable and frequently used microorganism due to its environment-friendly behaviour and ability to grow in almost any kind of conditions. Recently, it has been stated by NASA that the nutritional value of 1000 kg of fruits and vegetables equals to 1 kg of *Spirulina*. Even though it has all these advantages, cyanobacteria need to be explored more and more so that more fruitful results will come out.

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