Biotechnological Potentials and Role of Cyanobacteria in Agriculture and Industry

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Cyanobacteria (Blue Green Algae, Cyanophyceae or Cyanophytes)

A large and widespread group of microorganisms found in wide range of habitats including aquatic, terrestrial and extreme environments, show ability to perform oxygenic photosynthesis.

Morphologically diverse with unicellular, filamentous and colonial forms, contain different combination of photosynthetic pigments like chlorophyll a, carotenoids and phycobiliproteins, Depict variability in physiological and/or biochemical attributes

Exhibit widespread compatibility and adaptability to extremes of temperature, desiccation, illumination, radiations, salinity, pH, toxicants and nutrient availability

Play a significant role in –

  *Environmental management* – as soil conditioners, biofertilizers, ameliorants of degraded wastelands and polluted water bodies, and scavengers of heavy metals.

  *Bioindustry* - as a source of natural pigments, nutritional supplements, pharmaceuticals / drugs, Biofuel etc.
Applications in Biotechnology

- Agriculture
- Food and Feed
- Fuel
- Pharmaceuticals
- Aquaculture
- Value addition
- Bioremediation
Advantages of cyanobacteria

- Higher photosynthetic efficiency, faster growth and easily mass cultured.
- The biomass conversion efficiency is 18 percent as compared to only 6 percent for crops due to the microscopic size which increases the surface area.
- Can be grown round the year, require less area and require simple, easily available cheap nutrients. The entire biomass can be used without any processing.
- The versatile energy conversion, less capital investment, more production per unit area, non polluting, environmentally friendly and ecologically safe nature of the production unit make these as the most promising source of future.

Potentials as Food and Feed: Single Cell Protein, Excellent Digestibility, Provides essential amino acids, vitamins and minerals, Feed for fish, cattle and poultry

Anabaena flosaquae and Spirulina afford easily manageable sources of cheap protein, minerals and essential amino acids.
Important input for better crop nutrient management in soil
Diazotrophic cyanobacteria maintain and increase the soil fertility of rice fields. Reduce the nitrogenous consumption by 15-30 percent.

The heterocystous cyanobacteria (Anabaena, Aulosira, Calothrix, Cylindrospermum, Hapalosiphon, Nostoc, Scytonema, Tolypothrix and Westiellopsis) utilize molecular nitrogen and fix this up to ammonia under aerophillic conditions.

- Improve soil health by modifying the physical and chemical nature.
- These may increase the utilization of fertilizer nitrogen by partially reducing the losses through run-off, leaching and denitrification.
- Release amino acids like alanine, aspartic acid, glutamic acid, vitamin B₁₂ and auxin like substances which can also be beneficial for crop plants.
- Mucilage secreted binds the soil particles which increases the size of soil aggregates. This reduces soil compaction, increases pore size, aeration and water holding capacity.
- Can solubilize the insoluble phosphates in the soil.
- Growth has a buffering effect on soil pH.
- Reported to reduce the salinity in the soil leading to better crop response.
• Effectively utilized as soil inoculants. We must identify “Super strains”, performing the desired functions, grow them on a large scale and make quality inocula available on demand.

• Commercially viable method of producing clay based inoculum is available. BGA biofertilizer has composite culture of *Nostoc*, *Anabaena*, *Aulosira* and *Tolypothrix* sp.

• Growing the algae in separate raceways as unialgal cultures and mixing the fresh biomass with equal quantity of dried and finely powered clay has been advocated.

• The paste is sun dried, powered and stored in polythene bags at ambient temperature.

• The method ensures high titre value and longer shelf life. Such formulations perform additional functions

*Benefits of soil inoculation with blue green algae*

- Improves soil aggregation
- Nitrogen contribution
- Increases grain yield
- Increases soil organic matter content
- Can reduce weed population
- Increases P-availability
- Excretes plant growth regulators
- Increases soil microbial population
Production of Blue Green Algal Biofertilizer
Growth of Blue Green Algae in Rice Fields
Perfection of a protocol for the large scale multiplication of algae has made viable commercial production of the algal inoculum.
Flowchart of BGA Biofertilizer Production

Test Tube → Liquid Flask Culture

Quality Assessment

Production in Indoor tanks
Fresh biomass
Mixing with carrier
Packaging and sealing → Storage
<table>
<thead>
<tr>
<th>Place/Distt.</th>
<th>No. of trials</th>
<th>Average N saving (Kg/ha)</th>
<th>Percent increase in yield</th>
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<tr>
<td>Galand / Ghaziabad</td>
<td>15</td>
<td>25</td>
<td>8.2</td>
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<tr>
<td>Alipur block / Delhi</td>
<td>8</td>
<td>15</td>
<td>12</td>
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<tr>
<td>Rajlubhogipur /Sonepat</td>
<td>12</td>
<td>15</td>
<td>4.18</td>
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<tr>
<td>Pasina Kalan / Panipat</td>
<td>6</td>
<td>-</td>
<td>8.44</td>
</tr>
<tr>
<td>Asaundha / Jhajar</td>
<td>10</td>
<td>15</td>
<td>12.1</td>
</tr>
<tr>
<td>Asoda Todran / Bahadurgarh</td>
<td>10</td>
<td>-</td>
<td>19.48</td>
</tr>
<tr>
<td>Jakhoda / Bahadurgarh</td>
<td>10</td>
<td>-</td>
<td>12.26</td>
</tr>
</tbody>
</table>
Future Aspects

- Single commercially viable method
- Extensive field trials for awareness building
- Quality certification
- Off the shelf availability
- Region specific Biofertilizer inoculants
- Survival and evaluation of inoculant strains
- Assessment of total contribution
- Widening the scope of application
Symbiotic association of water fern *Azolla* with *Anabaena azollae* is exploited in crop production.

This association has trophic independence for carbon and nitrogen and provides 20-40 kgN/ha/season.

*Azolla* is used in rice cultivation by way of incorporating into the soil or grown as dual crop.
• Intercropping or dual crop method, 250-800 kg fresh *Azolla* fronds introduced in 1 hectare after rice transplantation, allowed to grow with the rice crop.

• Efficient phosphorus scavenging mechanism in *Azolla* helps in concentrating P from the surroundings.

• Used for human consumption in Vietnam and some other countries.

• Dried inoculum with viable spores enables transportation to the distant places.

• Introduction of biofertilizer concept (BGA and *Azolla*) in Indian Agriculture has reduced the pressure on the energy intensive fertilizer industry particularly for poor and marginal farmers.

• The technology is ecofriendly and sustainable.

Growth of *Azolla* in rice fields
**Spirulina**: Blue green prokaryotic, filamentous alga with closely coiled spirals of 300-500µm length, sometimes occurs as short, almost aseptate, loosely coiled or straight filaments. No sheath in trichomes, filaments filled with gas vacuoles. Human and animal food supplements produced primarily from two species *Spirulina platensis* and *Spirulina maxima*, found in most lakes and ponds.

Considered to be a low fat, low calorie, cholestorl free protein up to 65% protein with all essential amino acids. Has γ linolenic acid which dissolves fat deposits.

**Scientific classification**
- Domain: Bacteria
- Phylum: Cyanobacteria
- Class: Chrooobacteria
- Order: Oscillatoriales
- Family: Phormidiaceae
Beta Carotene
- Produces Vitamin-A
- Which is good for the eyes

GLA
- Controls cholesterol
- Improve skin tone

Antioxidants
- Slow down the Ageing process

Phycocyanin
- Strengthens the immune system

Proteins
- Vital for growth and development

Vitamin-B Complex
- For effective metabolism of nutrients

Iron
- Helps in the formation of haemoglobin

Calcium
- For healthy bones and teeth
Nutrients and Other Chemicals

➢ Proteins
Contains unusually high amounts of protein, 55 and 77% by dry weight. **Complete protein**, containing all **essential amino acids**, as compared to standard proteins from meat, eggs, or milk; Superior to all standard plant protein, such as **legumes**.

➢ Vitamins
Contains most vitamins in high quantities, but **richest** in vitamin A (in the form of β-carotene). Also has vitamin D, K, C, E, B1 (Thiamine), B2 (Riboflavin), B3 (Niacin), B6 (pyridoxine) and B12 (Cobalamin). The **bioavailability** of B12 in **Spirulina** is in dispute.

➢ Minerals

➢ Pigments
Diabetes
Hypoglycemic effects observed in studies with *Spirulina* fed diets in Japan. Administration of *Spirulina* brought about a significant decrease in blood sugar in non-insulin dependent diabetes (Dinesh Babu, 1989).

Hypcholesterolemic effects observed in rat experiments (Chen *et al.*, 1981; Kato *et al.*, 1984; Anusuya Devi and Venkataraman, 1983).

Antiallergic Property
Antiallergic protection by preventing release of histamines which contribute to allergy symptoms such as Runny Nose, Watery Eyes, Hives, Soft tissue swelling

Anemia
Rat experiments- An improved absorption of iron (Johnson, 1986)
Enhancement in Haemoglobin content by 21% (Takeuchi, 1978)

Arthritis
Patient suffering from rheumatoid arthritis-fed with *Spirulina fusiformis*–marked improvement and decrease in pain and swelling (Mohan 1992)

Antibiotic-Related Illness
*Spirulina* promotes the growth of *L.acidophilus* and other probiotics
Antiviral activity against Herpes, Influenza, Cytomeglovirus and Human Immunodeficiency Virus (HIV)
Foods rich in carotenes or vitamin A are associated with reduced risk of cancer. High level of chlorophyll may stop cancer (One Earth Herbal SourceBook).

Animals given chlorophyll supplement before exposure to cancer causing substance developed fewer tumours than those not given supplement (Oregon State University Study).

- Oral Cancer
  - In One study, 87 people who chewed tobacco developed leukoplakia. The lesions disappeared to some extent with *Spirulina fusiformis*.

  - 1-gm *Spirulina* capsule given daily for a year to 44 tobacco and betel nut chewers in India who had developed precancerous lesions in mouth. Complete regression of lesions in 45% people (Padmanabhan Nair- USDA-ARS Beltsville Human Nutrition Research Centre (MaryLand)).

  - Tumour inhibition and regression of buccal pouch cancer in hamster and human (Schwartz and Shklar, 1986)

  - Prevention of generation of malignancy.
Role of phycocyanins

- Selective inhibition of cyclooxygenase-2 (Reddy et al., 2000)
- Induces apoptosis in mouse in macrophage cell line RAW 264.7 stimulated with LPS (Reddy et al., 2003) & Rat histiocytoma cell line AK5 (Parthasarathy et al., 2003)
- Induces Apoptosis in K562 cells by cytochrome C release from mitochondria into cytosol.

Other Effects against

- Heart problems
- Hepatitis
- Pancreatitis
- Constipation
- Wound Healing
- Cataract
- Over weight
- Degenerative diseases
- Alzheimer’s and Parkinson’s disease
- Lead induced Lipid Peroxidation
<table>
<thead>
<tr>
<th>Uses based on scientific evidence in humans &amp; animals</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>C</td>
</tr>
<tr>
<td>Spirulina refers to a large number of &quot;cyanobacteria,&quot; or blue-green algae. Blue-green algae are found in the warm, alkaline waters of the world, especially of Mexico and Central Africa. Spirulina species are most often grown under controlled conditions and are subject to less contamination than non-spirulina species that may be harvested in nature. Preliminary study of people with type 2 diabetes mellitus reports that spirulina may reduce fasting blood sugar levels after two months of treatment. More research is needed before a firm conclusion can be drawn.</td>
<td></td>
</tr>
<tr>
<td>High cholesterol</td>
<td>C</td>
</tr>
<tr>
<td>In animal studies, spirulina has been found to lower blood cholesterol and triglyceride levels. Preliminary poor-quality studies in humans suggest a similar effect. Better research is needed before a firm conclusion can be drawn.</td>
<td></td>
</tr>
<tr>
<td>Oral leukoplakia (pre-cancerous mouth lesions)</td>
<td>C</td>
</tr>
<tr>
<td>Preliminary research has not clearly shown benefits of spirulina in the treatment of oral leukoplakia.</td>
<td></td>
</tr>
<tr>
<td>Weight loss</td>
<td>C</td>
</tr>
<tr>
<td>Spirulina is a popular therapy for weight loss, and is sometimes marketed as a &quot;vitamin enriched&quot; appetite suppressant. However, little scientific information is available on the effect of spirulina on weight loss in humans.</td>
<td></td>
</tr>
<tr>
<td>Chronic viral hepatitis</td>
<td>D</td>
</tr>
<tr>
<td>Preliminary study of spirulina for chronic viral hepatitis shows negative results.</td>
<td></td>
</tr>
<tr>
<td>Malnutrition</td>
<td>D</td>
</tr>
<tr>
<td>Spirulina has been studied as a food supplement in infant malnutrition. Spirulina does not seem to give added benefit over traditional renutritions, is more costly and is not recommended.</td>
<td></td>
</tr>
</tbody>
</table>

Key to grades

A: Strong scientific evidence
B: Good scientific evidence
C: Unclear scientific evidence
D: Fair scientific evidence
Standardization: There is no widely accepted standardization for Spirulina products.

Adults (18 years and older)
• Diabetes mellitus (type 2): 1 gram of Spirulina by mouth twice daily with meals has been studied.
• High cholesterol: 1.4 grams of Spirulina by mouth, three times daily with meals, for eight weeks has been studied.
• Oral leukoplakia (pre-cancerous mouth lesions): 1 gram of *Spirulina fusiformis* by mouth daily has been used for up to a year in one study.

Weight loss: 200 milligrams of Spirulina tablets by mouth three times daily, taken just before eating, has been studied.

Children (under 18 years old): Not enough scientific information is available to advise the safe use of spirulina in children

Side Effects and Warnings
- Few side effects have reported with Spirulina use. Most frequently reported adverse effects are headache, muscle pain, flushing of the face, sweating, and difficulty in concentrating. Described in people taking 1 gram of Spirulina by mouth daily. Skin reactions have also been reported.
- May be contaminated with heavy metals. **Liver damage** has been reported.
- The amino acid phenylalanine may cause an adverse reaction in people with the genetic condition **phenylketonuria** (PKU), and should be used cautiously.

Pregnancy and Breastfeeding
Enough information not there to recommend the safe use of spirulina during pregnancy or breastfeeding. In mice, diets containing up to 30% spirulina not reported to cause harmful effects to either the mother or the offspring. However, reliable human studies addressing safety during pregnancy or breastfeeding are not available.
# Spirulina Powder Analysis

<table>
<thead>
<tr>
<th>Physical properties</th>
<th>General analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Composition</strong></td>
<td><strong>Protein</strong></td>
</tr>
<tr>
<td>100%</td>
<td>55-70%</td>
</tr>
<tr>
<td><strong>Appearance</strong></td>
<td><strong>Carbohydrates</strong></td>
</tr>
<tr>
<td>Fine Powder</td>
<td>15-25%</td>
</tr>
<tr>
<td><strong>Colour</strong></td>
<td><strong>Fats (Lipids)</strong></td>
</tr>
<tr>
<td>Dark Blue - Green</td>
<td>06-08%</td>
</tr>
<tr>
<td><strong>Odour &amp; Taste</strong></td>
<td><strong>Minerals (Ash)</strong></td>
</tr>
<tr>
<td>Mild like Sea-Weed</td>
<td>07-13%</td>
</tr>
<tr>
<td><strong>Bulk Density</strong></td>
<td><strong>Moisture</strong></td>
</tr>
<tr>
<td>.35 to .60 kg/lit</td>
<td>03-07%</td>
</tr>
<tr>
<td><strong>Particle Size</strong></td>
<td><strong>Fibre</strong></td>
</tr>
<tr>
<td>64 mesh through</td>
<td>08-10%</td>
</tr>
</tbody>
</table>
Growth of *Spirulina platensis* and its mass production for possible utilization as nutritionally rich formulations (a) *Spirulina* biomass in liquid culture (b) Photo micrograph showing *Spirulina* filaments (c) Growing *Spirulina* in tubs (d) Buckets with *Spirulina* (e) Growth units of *Spirulina* covered with muslin cloth (f) Hapkins flask (g) Culture room
Low cost cultivation

Natural saline water → Cultivation pond -Cement tank → Raw sewage

Harvesting

Biomass 200m² will produce about 100kg Dry biomass/month

Crude protein

Press Reduce to thick paste through machine → Drying packaging (Pills, Powder, chips, Noodle like)

Enzymes, Cytochromes

Other chemicals

Pigments

Chlorophylls carotenes, Phycobilins etc.
Raceways with paddle wheel arrangement for mass multiplication of microalgae in CCUBGA, IARI in glass house facility
In cemented tank or raceways lodged in a covered space for round the year production. The ratio of the inoculum in raceway is kept 1:10. The culture is filtered through cheese cloth and filtrate is drained back to raceway when the growth of the alga slows down than appropriate quantities of fresh nutrients may be added.
Commercial production of *Spirulina*
Open raceway paddle wheel mixed ponds now used by 98% commercial microalgae production (Shown: Spirulina farm, Earthrise Co. CA)
Spirulina Culture Expansion
(Earthrise Farms)
Spirulina and Haematococcus Ponds at Cyanotech Corp. in Hawaii
Eni Project (Monterotondo, Italy)
Compared PBRs & ponds using flue gas CO2
Mass multiplication in a photobioreactor at CCUBGA, IARI
YASH PHARMA INTERNATIONAL [India] 
Spirulina, organic spirulina

Bluray Nutritional Products [India] 
spirulina tablets, spirulina powder

MODI HERBALS [India] 
Health Food

Elken Spirulina is Certified Organic

Wonder Herbs. Pvt. Ltd [India] 
Spirulina Spray Dried Powder

Tan-hoard Exports [India] 
Spirulina Capsule
Commercial preparations like Spiruvit and Sunnova as capsules containing dry powder of *Spirulina* are available. These are recommended as antioxidants, probiotics and neutriceuticals.
Hydrogen production from cyanobacteria

Many have the ability to produce hydrogen under a range of cultural conditions:

- Unicellular non-diazotrophic *Gloeocapsa alpicola* shows increased hydrogen production under sulphur starvation.

- *Spirulina platensis* can produce hydrogen in complete anaerobic and dark condition.

- *Anabaena cylindrica* produces hydrogen in an argon environment in light limited conditions.

Phtobioreactor for algal H2 production and system for gas collection.
Environmental protection

- Selective uptake, accumulation and biodegradation of pollutants by algae are important in remediation.
- *Microcystis, Oscillatoria, Lyngbya* can act as bioindicators of degree and type of pollution.
- Fast growing algae are employed to harvest nitrogen and phosphorus from waste waters.
- These can reduce nitrate content in the ground water.
- Can be used in turf grass treatment for checking the formation of black layer formed due to the accumulation of metallic sulphides.
- Anaerobiosis is reduced by liberation of oxygen during photosynthesis. Algae effective for the purpose.
- The spacecraft can be provided with oxidation ponds which can convert waste into consumable forms and provide oxygen to the astronauts.
• Biological reclamation of sewage undertaken at NEERI (National Environmental Engineering Research Institute, Nagpur). The dominant alga in oxidation ponds reported are *Oscillatoria chalybea*, *Anacystis* and other microalgae.

• Algae can immobilize heavy metals from aquatic systems.

• *Aphanocapsa* and *Oscillatoria* reduce the levels of total dissolved substances and calcium from the industrial effluents.

• High degree of accumulation of radio-nuclide on algal biomass has been observed. *Nostoc* can fix 40% of radio cesium and cobalt.

• Algae can metabolize bicarbonate and nitrates in waste waters. This can increase the pH to 10 or more, which accelerates autoflocculation of algal biomass incorporating bacteria and polyvalent cations as copper, chromium, iron, manganese, lead, strontium and zinc with calcium and magnesium, which settles to the bottom.

• Algal biomass can be collected in the production of combustible gases and organic manure.
Microalgae as biocontrol agents

• ‘Insect’ a commercial bioinsecticide sold in USA is prepared from the dead biomass of algae. Properly crushed and processed pieces are mixed with bait. These penetrate and scratch the waxy covering on the body of the insects leading to its death due to dehydration.

• Attempts made to introduce endotoxin genes from *Bacillus sphaericus* in *Chlorella* which can be used as potential bioinsecticide. *Phormidium, Plectonema, Oscillatoria* can receive endotoxin genes which can have implications in controlling mosquito breeding.
Role in Pharmacy

- An anticancer factor identified in *Scytonema* sp., *Phormidium tenue* and *Anabaena variabilis*.

- Two new *Lyngbya* toxins very similar to teleocidin A-1, isolated from marine *Lyngbya majuscula* have been indicated in cancer treatment.

- Role as sources of many secondary metabolites having antibacterial, antifungal, antiviral and antitumor activities reported.

- Production of tylootoxin from *Scytonema ocellatum* which depolymerised actin to disrupt cell division and drug holds promise in cancer treatment.

- Chlorine containing β-carbolines (bauerines A-C) from terrestrial blue green alga *Dichothrix baueriana* GO-25-2. These alkaloid showed activity against simplex virus Type 2.
Saviours of Green House Effect

- Can fix 3.2X1010mt of CO₂. The CO₂ absorbed at plasma membrane can be converted into HCO₃, translocated through the cytosol to be accumulated at carboxysomes and converted back to CO₂ by carbonic anhydrase. The cyanobacteria which hold promise of acting as sinks for CO₂ is Oscillatoria.
- Efficient scavengers of CO₂ with increased photosynthesis, can reduce CO₂ levels and mitigate dangers of green house effect, provide useful algal biomass which can be put in variety of uses.
Health food for future

thank you