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Development of Biofertilizer from Industrial Wastewater

Authors: Namratha P. M. [1#], Vaishnavipriya K, Yogavarshini S., Gautham S., Mari Maheshwaran B.

[#]**Corresponding Author:** Namratha P. M.

Assistant professor (Biotechnology),

Shri Shakthi Institute of Engineering and Technology, Coimbatore 62

Author affiliations

Authors name	**Designations and Highest degree(s)**	**Email address**	**Affiliations**
Namratha P. M.[1]	Assistant professor (Biotechnology), M. Tech Biotechnology	namrathapm@mail.comORCID:0009-0002-9257-779X	Shri Shakthi Institute of Engineering and Technology, Coimbatore
Vaishnavipriya K.	Student: B. Tech Biotechnology	kubervaish@gmail.com	Shri Shakthi Institute of Engineering and Technology, Coimbatore
Yogavarshini S.	Student: B. Tech Biotechnology	yogavarshinis406@gmail.com	Shri Shakthi Institute of Engineering and Technology, Coimbatore
Gautham S.	Student: B. Tech Biotechnology	Alex869487265027@gmail.com	Shri Shakthi Institute of Engineering and Technology, Coimbatore
Mari Maheshwaran B.	Student: B. Tech Biotechnology	maheshwaranmari12@gmail.com	Shri Shakthi Institute of Engineering and Technology, Coimbatore

Keywords: Wastewater Sample, Algal sample, Urea, Sodium bicarbonate pellets.

Abstract

Soil degradation, overuse of chemical fertilizer, and biodiversity loss are serious problems challenging the sustainable development of modern agriculture. In recent years, owing to the advantages of algae biotechnology in nutrient recovery and soil improvement, the integration of algae-based wastewater remediation and algal bio-fertilizer production has emerged into the limelight. Algae including microalgae and cyanobacteria could effectively remove a portion of nutrients in wastewater, and at the same time, produce biomass. Therefore, agriculture-related wastewater can be a good medium to cultivate algae for bio-fertilizer production. Identification and characterization of the algae grown has to be done. Invitro screening of the algal community will reveal the nutrients produced by individual species and the amount of additional nutrients to be added for refining the growth of plants. The algal biofertilizers can increase crop yield by 1014% and unlike chemical fertilizers they are eco-friendly. Since the algae is grown in agriculture wastewater media cost is reduced and hence low-cost input in the biofertilizer production.

Introduction:

Biofertilizers are live microorganisms that improve the chemical and biological aspects of soils, restore soil fertility, and encourage plant growth. Plants require nitrogen to flourish, and a deficiency in this nutrient can be corrected by applying enough fertilizer. Biofertilizers are substances that contain microorganisms, which when added to the soil increase its fertility and promote plant growth. Organic biofertilizers are natural substances containing living microorganisms that enhance soil fertility and plant growth (Chatterjee et al., 2017). They promote nutrient availability by fixing nitrogen, solubilizing phosphorus, and producing growth-promoting substances. Popular examples include rhizobacteria, mycorrhizal fungi, and nitrogen-fixing bacteria. They offer sustainable alternatives to synthetic fertilizers, supporting eco-friendly agriculture. Biofertilizers are substances that contain microorganism's living or latent cells. Biofertilizers increase the nutrients of host plants when applied to their seeds, plant surface or soil by colonizing the rhizosphere of the plant. Biofertilizers are more cost-effective as compared to chemical fertilizers (Ammar et al., 2023).

Algae play an important role in agriculture as biofertilizers. They provide environmentally friendly organic fertilizers at a cheaper price. Algae play an important role in the maintenance of the natural habitat of the soil, increase in crop yield, replacement of chemicals such as nitrogen and phosphorus, enhancement of plant growth, and help to retain water during drought (Win et al., 2013). Farmers commonly use red and brown algae, as both species are rich in potassium. This potassium works to encourage root growth and improves resistance to drought conditions. Blue-green algae also contain a good amount of potassium and have anti-fungal properties. When you compost or dry algae, they effectively bind the soil together and increase its moisture-retention capacity (Iyer et al., 2015). Algae also improve soil aeration, which lets roots absorb nutrients more efficiently. Algal strains used for the study

- Chlorella vulgaris
- Spirulina platensis
- Azolla Anabaena
- Gracilaria verrucosa

Chlorella Vulgaris

It is a species of single-celled green algae belonging to the genus Chlorella. Characterized by its spherical cell structure, Chlorella vulgaris is recognized for its rich nutritional content, including proteins, essential amino acids, vitamins, minerals, and antioxidants. It is often utilized as a dietary supplement due to its potential health benefits. Additionally, Chlorella vulgaris has been studied for applications in biofuel production and wastewater treatment, highlighting its versatility and significance in various fields.

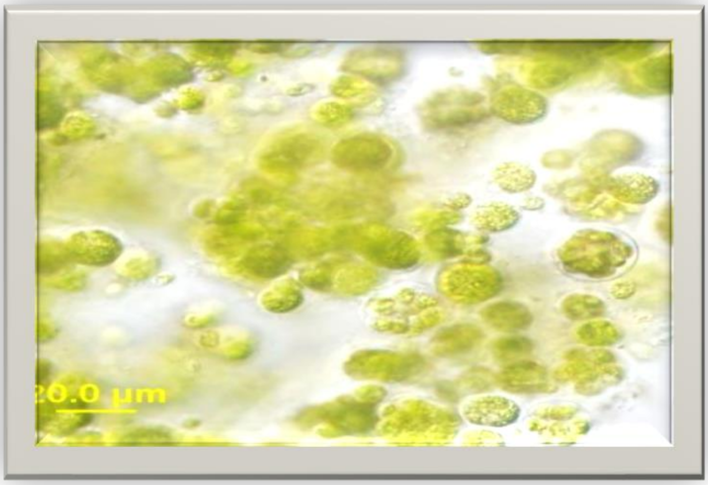

Fig No 1: Chlorella Vulgaris

Spirulina platensis

It is a species of cyanobacteria, commonly referred to as blue-green algae. Spirulina is recognized for its unique spiral-shaped filamentous structure and its vibrant blue-green color. It is often cultivated for its exceptional nutritional content, including high levels of proteins, vitamins (especially B vitamins), minerals, and other bioactive compounds.

Fig No 2: Spirulina platensis

Azolla Anabaena

Azolla is a genus of small aquatic ferns, and Anabaena is a genus of nitrogen-fixing cyanobacteria. The Azolla-Anabaena symbiosis refers to the mutually beneficial relationship between these two organisms. Anabaena, housed within the cavities of Azolla leaves, is capable of nitrogen fixation. It converts atmospheric nitrogen into a form that can be utilized by plants, thus enhancing the nitrogen content in the surrounding environment.

Fig No 3: Azolla Anabaena

Gracilaria verrucosa

It is a species of red macroalgae, belonging to the genus Gracilaria. Commonly known as "Ogo" in Hawaiian and sometimes referred to as "Cottonii" in certain regions, it is characterized by its bushy and branching morphology. Typically found in shallow marine environments, Gracilaria verrucosa attaches to rocky substrates or other submerged surfaces in tropical and subtropical coastal waters.

Fig No 4: Gracilaria verrucosa

Most cyanobacteria can fix nitrogen from the atmosphere and several species including Anabaena sp., Nostoc sp., and Oscillatoria angustissima is known to be effective cyanobacterial-based biofertilizers (Ghosh et al., 2016). Acutodesmus dimorphus, Spirulina platensis Chlorella vulgaris, Scenedesmus dimorphus, Anabaena azolla, and Nostoc sp. are some of the green microalgae and cyanobacteria species that have been successfully used as biofertilizers to boost crop growth. Also, Chlorella vulgaris is one of the most used microalgae in biofertilizer studies.

In greenhouse-grown garden pea and field plots of spring wheat, researchers tested the impact of algae addition on plant development, nutrition, and soil physical and chemical features, using five species of algae that contrasted with the basic makeup. The cyanobacterium Arthrospira platensis (Spirulina), unicellular green algae Chlorella sp., red seaweed Palmaria palmate, and brown seaweeds were among them (Laminaria digitata and Ascophyllum nodosum). Chlorella and Spirulina enhanced total nitrogen and accessible phosphorus in the soil, and Spirulina improved soil nitrate levels (Bhattacharjee et al., 2014). The inorganic (NH + 4 and NO3) concentrations in the soil were significantly improved by Palmaria palmata and Laminaria digitata. Chlorella sp increased total P, N, and C in the soil, as well as accessible P, NH + 4, N, and pea production. Algae additions had little effect on the water-stable aggregates in the soil. Chlorella sp., Spirulina, P. palmata, and L. digitata all enhanced soil inorganic nitrogen concentrations in the field.

Materials and Methods:

Chemicals

Sample water, Algal samples: Chlorella vulgaris, Spirulina platensis, Azolla Anabaena, Gracilaria verrucosa, Urea, Sodium bicarbonate pellets.

Methods

Identification of algal sample:

Identifying algae involves a combination of visual observation, microscopic examination, and sometimes biochemical analysis. Here are general steps for algae identification:

Fig No 5: Industrial wastewater sample collected

Macroscopic Characteristics:

Color: Observe the color of the algae, which can range from green and brown to red or other hues.

Form: Note the general form of the algae – whether it's filamentous, colonial, unicellular, or multicellular.

Microscopic Examination:

Use a microscope to examine the cellular structure of the algae. Note the presence of chloroplasts, cell wall characteristics, and any unique structures. Measure cell size and shape, and observe the arrangement of cells in colonies or filaments.

Pigment analysis:

Determine the pigments present in the algae, such as chlorophyll, carotenoids, or phycobilins.

Habitat and Ecology:

Consider the environment where the algae are found. Different algae thrive in various habitats like freshwater, marine environments, or moist terrestrial areas.

Nutrient Requirements:

Understand the nutrient requirements of the algae. For instance, some algae thrive in Nutrient-rich waters, while others prefer nutrient-poor conditions.

Taxonomic Keys:

Use taxonomic keys specific to algae for further identification. These keys typically guide users through a series of questions or characteristics to narrow down the identification.

DNA Analysis (Advanced): In advanced cases, DNA analysis can be employed for precise identification. This involves sequencing specific regions of the algae's DNA and comparing it to known sequences in databases.

Consulting Algal Experts:

Seek guidance from experts or taxonomists specializing in algae. They may provide valuable insights based on their expertise.

Online Resources:

Utilize online databases and resources that provide images and descriptions of various algae species. These can serve as references during the identification process.

Filtration

Selection of Whatman Filter Paper:

Choose the appropriate grade and pore size of Whatman filter paper based on the size of particles you need to filter. Different grades are available for varying filtration needs.

Filtration Setup: Set up a filtration apparatus, which typically includes a filtration funnel, filter flask, and a vacuum pump or water aspirator. Ensure that all components are clean and assembled correctly.

Wetting the Filter Paper:

Wet the Whatman filter paper with a small amount of the wastewater or an appropriate solvent. This helps create a seal and prevents air bubbles from forming.

Filtering Wastewater:

Pour the wastewater through the wetted Whatman filter paper in the filtration funnel. Apply a vacuum to assist in the filtration process. The liquid portion of the wastewater will pass through the filter paper, leaving the solid particles behind.

Transfer of Filtered Water:

Collect the filtered water in the filter flask or another container, free from the separated particles.

Fig No 6: Filtered wastewater sample

Preparation of sample water:

Collection of waste water samples from different sources, Strain the waste water by using Whatman Filter Paper. Check the pH of the water sample by using pH paper. Culturing of algal samples present in the wastewater by providing optimized culture conditions. Developing and optimizing the biofertilizer using the algal culture produced (32^0C, pH 9, stirring and ample light).

Fig No 7: Spirulina culture plate

Fig No 8: Inoculated wastewater sample

FLOWCHART:

Waste water sample

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Filtration

↓

Inoculation

↓

Culturing the algal sample

↓

Growth of algae in water sample

↓

Making Biofertilizer in liquid form

Results:

Preparing Biofertilizer from Wastewater

Identification of algal sample:

The wastewater sample was incubated under algal growth conditions for about a week without any inoculation to check whether it had any algal sample and whether it would grow on its own if provided with proper growth conditions. The microscopic, visual, and macroscopic examination of the wastewater sample without inoculation of any algal sample, didn't show any growth. The nutrient requirements need to be analyzed for further study.

Inoculation of algal cultures to the wastewater samples:

The study was conducted first using Spirulina platensis as the algal culture. Collection of wastewater samples from different sources, Strain the waste water by using Whatman Filter Paper. Check the pH of the water sample by using pH paper. Culturing of algal samples present in the wastewater by providing optimized culture conditions. Developing and optimizing the biofertilizer using the algal culture produced (32^{0}C, pH 9, stirring and ample light).

Fig No 9: Industrial wastewater sample after incubation with spirulina for algal growth

Fig No 10: Development of Biofertilizer

Discussion

The spirulina culture was produced in the lab scale using wastewater samples collected without adding any extra nutrients. The required growth conditions with wastewater substrate gave good spirulina growth. This development of biofertilizers from cheap substrate would help develop good crop productivity in minimum land requirements which in turn is a good sustainable environment achievement. The lab study must be scaled up to pilot scale and the growing conditions has to be optimized as further study**.**

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Author Information

Sri Shakthi Institute of Engineering and Technology, Coimbatore 641062, Tamilnadu, India

Author Contributions

Namratha P M has performed the literature search, compilation, drafting, preparation or writing, and revision of the manuscript. Vaishnavipriya K, Yogavarshini S., Gautham S., Mari Maheshwaran B. experimented.

Corresponding author

Correspondence to Namratha P. M.

Ethics declarations

Conflicts of interest

The authors declare no conflict of interest.

Ethics approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate and consent to the publication

Not applicable

Data availability

Data availability and sharing do not apply to this article as no datasets were generated or analyzed during the study.

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