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Microbial Exploitation of Biofertilizer, Pullulan and Biochar from Floral Waste

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Abstract

In diversified India, there are different traditions and cultures and their spirituality to believe in God is unconditional. People used to go temple and offer several things to God, and mainly the item they offer is the flower. Heaps of flowers flooded in temples are served as waste in nearby areas, or they dumped it into water. Flower waste has abundant properties with several nutrients (macro as well as micro) in them. In this article, we have emphasized the production and application of fertilizer, Pullulan and Biochar with the help of microbes present on floral waste enriched samples. Test samples were collected in the nearby areas of the temple, and after collection, serial dilution of the enriched sample was prepared. 1mL of each serial dilution were outspread on sterile nutrient agar plates, and bacterial isolation was done. Properties of degradation of soil isolates were evaluated. Flowers collected were then dried, and 1% was then transferred in minimal medium (without C source). Streaking was carried out, and isolated possessing tremendous growth was choosen for the development of the consortium. Different combinations of soil isolates were used. Five per cent inoculum of various consortia was spread on flower waste chambers. It was incubated aerobically. Degradation was analyzed after a specific time interval. The microbial consortium supports minimizing the time required for degrading an enormous amount of flower waste. With this perspective, we can prepare good quality Biopolymer Pullulan and Biochar without causing any harm to the environment.

Keywords: Flower waste; Isolation; Degradation; Pullulan; Biochar; Applications

Introduction

Flower waste disposal is a central problem in the world. Diversity in the content of waste creates problems in its reduction. Safe disposal of floral waste has been a matter of disquiet for the temple management. The floral waste is directly disposed of into the rivers, oceans, etc., which harms the water quality and the living organisms present in the water. Flowers come as waste from hotels, wedding ceremony gardens, worship places, and various civilizing and sacred ceremonies, making them a usual floral waste source. Flowers are considered holy entities and hence are offered by pilgrims to their idols. Every day these flowers provided by the devotees in temples are left unused and therefore become waste. This flower waste gets accumulated at religious sites like Temples, Mosques and gurudwaras due to several spiritual practices and is also generated in places like residential areas, community centers, etc.

Floral waste degradation is a prolonged process compared to kitchen waste degradation [1]. By keeping this in mind, we have attempted to develop consortia to degrade the floral waste. The exploitation of the metabolic adaptability of microorganisms is advantageous in biological waste treatment. This led to a shortening of the time for the production of pullulan and Biochar.

Materials and Methods

Biofertilizer production

Biofertilizer production is a needy and valuable product for the cultivation of crops in recent times.

Sample collection and screening of microbes: Floral waste enriched soil samples were collected from the temple area. 10gm of soil sample were taken into 250ml of the conical flask and put on 90ml of sterile distilled water from sample soil. The flask was agitated for 10min on a rotary shaker. 1mL suspension was added to ml of via and shaken for 2min. Serial dilution technique was done if this suspension was expanded on flower waste agar plates up to 10-7 an aliquot (0.1ml). Incubation of plates performed for 24hrs at 37˚C. Different characteristics of colonies after incubation were detected, such as shape, size, elevation, surface, margin, colour, odour, pigmentation, etc. and gram's staining. Eight bacterial colonies were selected for flower waste degradation from all bacterial colonies grown on medium [2,3].

Development of Consortium: Different combinations of eight bacterial isolates were produced and utilized for biofertilizer preparation. Among these, the combination giving rapid degradation of flower wastes was selected for the consortium preparation. An optimal growth from 24hrs old bacterial culture of different organisms in a fixed variety was inoculated in minimal broth containing flower waste. The broth was incubated at 37^0C for 48hrs. After incubation, this broth was used as a consortium, and then 20% (v/w) of this consortium as inoculum was added to the flower waste [2,4].

Designing of degradation chamber: All organic material wastes available in the form of flowers were collected. Crushing of flower

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Figure 1: Sample Collection from Temple sites.

Table 1: Pullulan Production Efficiency of Some Reported Strains [11].

Serial No	Source Microorganisms	Pullulan Concentration
1	*Aureobasidium pullulans* ATCC42023	3.21±0.35 g/L
2	*Aureobasidium pullulans* IMI145194	13g/L
3	*Aureobasidium pullulans* HP-2001	5.5g/L
4	*Aureobasidium pullulans* FB-1	44.7g/L
5	*Aureobasidium pullulans* P 56	31.3g/L
6	*Aureobasidium pullulans* NCIM 976	23.6g/L
7	*Aureobasidium pullulans* HP-2001	11.49g/L
8	*Aureobasidium pullulans* ATCC201253	23.1g/L
9	*Aureobasidium pullulans* SZU1001	25.6g/L
10	*Aureobasidium pullulans* LDT-1	31.25g/L
11	*Rhodotorula bacarum*	59.0g/L
12	*Pullularia pullulans*	70.0g/L
13	*Aureobasidium pullulans* SK 1002	30.28g/L
14	*Aureobasidium pullulans* CJ001	26.13g/L
15	*Aureobasidium pullulans* RBF4A3	70.43g/L
16	*Aureobasidium pullulans* NPM2	25.1g/L
17	*Aureobasidium pullulans* FB-1	23.1±0.02 g/L
18	*Aureobasidium pullulans* RG-5	37.1±1.0 g/L

waste done by a crusher machine before being piled. Small size compost material is delicate and decomposes fastly. All the ingredients are mixed all together. These grind particles mix with a small amount of soil and blend the mixture equivalently. Two glass chambers with dimension 10.5×10×5.5cm were selected. In the first layer of coconut coir of height 2 cmat the bottom of the chambersaerobic conditions conserved. The coconut coir layer was covered by garden soil (2cm). The third layer was prepared by using flower waste inoculated with a 20% consortium. Alternative layers of soil and flower wastes inoculated with consortium were given as above. The second chamber was used as a control chamber using flower waste without consortium by following the exact procedure [5,6]. After absolute degradation of flower wastes, the degraded material is utilized as a biofertilizer.

Chemical analysis: Degradation completed then samples testing was done for pH, total minerals and N, P, K content. The total nitrogen, phosphorus and potassium (NPK) in keeping to the content of the following method of samples were investigated; using the macro Kjeldahl system; nitrogen was set on by the acid combustion elemental analysis method [7]. The phosphorus, potassium, and micronutrients were processed using the acid digestion method and investigated spectrophotometrically using the EPA technique [7]. The pH value was estimated in a 5-fold dilution of distilled water equilibrated with the sample for an hour with a pH meter. C% and N% was analyzed by the APHA method (APHA, 2005).

Efficacy of biofertilizers: The potential for the biofertilizers was carried out for several days. The soil for this experiment was garden soil having neutral pH and moisture content. A local species of wheat, green gram and Jowar were utilized as test plants. The experimental design was a completely randomized design with two replicates. Two sets of pots were developed for each test plant, one using prepared biofertilizer and another without using biofertilizer as control. Then fix the measured amount of good qualities seeds of all test plants were planted in corresponding pots. The pots were then watered daily for seven days. During harvesting, the plants were cautiously uprooted from each pot, and the plant height was recorded [8].

Pullulan production

The sample collection process is the same as biofertilizer production, but after having bacterial strains, further processing is done by solid-state fermentation diluted with deionized water. After fermentation, we get biomass, which is separated by Centrifugation or Filtration. After centrifugation, we get biomass and supernatant, activated charcoals or alcohols separated biomass as waste and supernatant with melanin with a combination of salts. Now the supernatant was precipitated and purified by Ultracentrifugation or Ion-exchange Chromatography. Finally, we get a purified Pullulan [9].

Biochar production

Sample collection was done, and after that, segregation was performed, which form different combinations of agitated piles using CCD-RSM (Composite central design- Response surface methodology). There is a decrease in size in the initial days of composting; to optimize the properties, we measure various parameters like pH, temperature, electrical conductivity, total organic carbon and nitrogen, nutrients, and C/N ratio. After optimization, we get a final compost, i.e., Biochar (rich in carbon).

Application of Biofertilizers, Pullulan and Biochar: Various applications are employed for small and large-scale industries and utilized as a good source of commercialization.

- Biofertilizers are extensively used in Agriculture markets, as they are eco-friendly and can be easily replaced by harmful chemical fertilizers.
- Pullulan is mainly used in food, pharmaceuticals, biomedical industries.
- Biochar has multifunctional values that include the soil amendment to improve soil health,
- Nutrient and microbial carrier,
- Immobilizing agent for remediation of toxic metals and organic contaminants in soil and water,
- The catalyst for industrial applications, porous material for mitigating greenhouse gas emissions and odorous compounds,
- Nutrient intake efficiency and Feed supplement to improve animal health consequently, the productivity [10].

Conclusion

The floral waste acted as a hub for the bacterial consortium and was used effectively by several preliminary techniques to produce biomaterials like biofertilizers, biopolymer (Pullulan), Biochar. Further, some parameters were analyzed to check the optimization studies of biomaterials. Validation experiments were performed and the results obtained showed the productions of multiple biomaterials viz; biofertilizers, pullulan and Biochar.

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