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REVIEW ARTICLE **OPEN ACCESS**

Low Cost Natural Media for Biomass Generation of Agriculturally Important Microbes

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ABSTRACT
UN projects world population to reach 8.5 billion by 2030 and 11.2 billion in the year 2100. The world will require an additional 1.3 billion tonnes of food (an increase of 50%) to feed the people. Crop production will have to focus on increasing efficiency of production by improved varieties, decreased use of chemicals, improved agricultural practices, judicious use of natural resources etc. One agricultural practice which will make crop production sustainable and also enhance yield is use of agricultural bio-inoculants i.e. biofertilizers. Metabolically active microbes provide nutrient and also protection against plant pathogens. Biomass generation for biofertilizer production is key to the success of biofertilizer production. Generally, microbial biomass is produced by using synthetic/semi-synthetic microbiological medium which adds to the cost of biomass production. Successful commercial and economical biomass production can be enhanced by using various natural substrates like agro-waste, molasses, sugarcane bagasse, dairy wastewater sludge as a growth medium. Few natural media that can be potentially exploited for commercial production of biofertilizer are outlined in the manuscript.
Keywords: *Microbial biofertilizers- uses and mode of application, biofertilizer production, natural media.*

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INTRODUCTION

Due to continuous cropping without recycling organic residues and over application of nutrients, decline in soil organic matter occurs. It also impairs soil health. Principle among them is the depletion of soil nutrients which has also increased cost of cultivation incurred due to large amounts of chemical fertilizer's usage. Rampant use of chemical sprays has also affected microbial ecology of the soil. This excessive use of chemical fertilizers causes serious environmental and health issues [1]. These problems include water, soil and air pollution, toxicity, production of carcinogenic substances in the environment. These chemical fertilizers also results in destruction of essential organisms and eco-friendly insects like pollinators, weed killers etc. It also increases the crop susceptibility to diseases and also acidification or alkalization of the soil [2].To mitigate these ill-effects, use of organic fertilizers is being popularized [1,2].

Beneficial plant-microbe interactions that promote plant health are essential for sustainable agriculture [3]. Biofertilizers are micro-organism based fertilizers which hold promise in sustaining agriculture. They are substances containing living micro-organisms which have the ability to colonize plant surfaces and rhizospheric soil. This in turn improves plant growth by increasing the supply or availability of primary nutrients to the host plant. In this regard, legume-rhizobia interaction for mutual benefit of both is very well documented. Biofertilizers for cereals crops as Wheat and Maize have also found wise application [4].

The EU "Common Agricultural Policy" promotes use of bio based products along with organic farming. Government of China has undertaken initiatives to educate farmers regarding use for biofertilizers. Restraining factor however, is easy availability of chemical fertilizers due to high number of production facilities especially in countries like China, India and Australia (www.grandviewresearch.com/industry-analysis/biofertilizers-industry). Europe and rest of the world countries especially Latin America are the two top consumers of biofertilizers in the world. Growth is particularly high in emerging countries such as China, India because of rising awareness among the farmers about the hazards of chemical fertilizers as well as the higher costs of chemical fertilizers (www.marketsandmarkets.com/Market-Reports/ compound- biofertilizers-customized-fertilizers-market-856.html).

ADVANTAGES OF USING BIOFERTILIZERS

Biofertilizers have numerous advantages as, it provides nutrients and growth promoting substances to plant at very low cost; fixes atmospheric nitrogen; aids in mineralization of plant nutrients like conversion of insoluble form of phosphate into soluble form; increases crop yield and improves soil health, retards or inhibits the growth of plant pathogens. Biofertilizers are required in small quantities which makes their use cost-effective and lowers overall cost of cultivation [5,6,7,8,9].

Biofertilizers are environment friendly alternative to chemical fertilizers. They enrich soil microflora and contribute towards sustainability of agriculture. A high dose of micro-organisms is required to be cultivated under suitable conditions for biofertilizer production. Usually a count of nearly one billion cells per unit of medium should be applied as biofertilizer to obtain the desired benefit [10]. Agricultural bodies recommend *Rhizobium* spp, *Bradyrhizobium* spp, *Mesorhizobium* spp, *Pseudomonas* spp, *Bacillus* spp, *Azotobacter* spp, *Azospirillum* spp as biofertilizers for legumes, cereals, cash crop and vegetables [11].

MODE OF APPLICATION

Biofertilizers can be applied either to the seed, seedling or the soil. For seed treatment- a slurry of one packet of biofertilizer of 250g is made in 500 ml of water. It is then applied on the surface of the seed which is then spread on clean cemented floor for drying in the shade. These seeds should be sown immediately. Seedling root dipping method is useful where the transplantation of seedling is required. It is ideal for vegetable crops. The inoculants suspension is prepared in the ratio of 1:10 using water. Roots of the seedling are dipped in this suspension for 30-45 minutes. Seedlings are then taken out from the suspension and transplanted at the earliest. For soil application-3-5 Kg biofertilizer inoculant is mixed with approximately 50 Kg of farm yard manure. It is then broadcasted over the field where sowing is to be done.

PRECAUTIONS DURING USE

Some precautions need to be taken during use of biofertilizer. Since plant-microbe association is specific, a biofertilizer recommended for a particular crop should be used for that crop only. Packet should be used before date of expiry and kept away for sunlight or heat during storage. Biofertilizer treated seeds should not be dried in the sun and should be sown immediately after treatment. Benefit of biofertilizer is lost when it is used along with chemical insecticides or pesticides.

NATURAL MEDIA FOR BIOFERTILIZER PRODUCTION

Biomass production of agriculturally important bacterial bio-inoculants is largely carried out using semi-synthetic microbiological media which forms major expense of this activity. Biofertilizers produced by government/cooperatives is averagely priced. The price of biofertilizer produced by private agro-industry is nearly ten times higher [4]. Cost-effective bacterial biomass generation is very crucial for viability of this task. Use of agro-waste can reduce the cost of production resulting in availability of a cheap biofertilizer product to the farmers and contributing towards sustainability of agriculture. In some biofertilizer production units, the substrate used for bacterial biomass generation is natural media, the composition of which is not in the public domain (personal communication).

Various natural media can be used for biomass generation [12,13,14]. Bioconversion of lignicellulosics residues to hexose and pentose sugars has been reported by many workers in India and abroad. Paddy straw is cheaply and locally available agro-waste. A number of workers have reported utility of paddy straw/pretreated paddy straw as substrate for fermentation as is evidenced by the quantum of literature available. These sugars can be potentially used as substrate for generating bacterial biomass and also resulting in synthesis of valuable by-products as organic acids, amino acids, vitamins, bacterial and fungal polysaccharide, ethanol and enzyme production [15,16,17,18,19,20,21,22]; for paper manufacturing; for compost making; for cultivation of edible fungi [23,24,25] and also used as animal feed.

Pretreated lignocellulosic biomass yields black liquor which is source of carbon for industrial processes [26]. Fermentable sugars generated after pretreatment of paddy straw are arabinose, xylose, glucose which are reported to support the growth of *Bacillus* spp, *Pseudomonas* spp, *Azotobacter* spp and also other bacteria and fungi. Pentoses in paddy hydrolysate have been demonstrated to support the growth of *Bacillus* spp and *Pseudomonas* spp for polyhydroxy butyrate (PHB) production - *Bacillus firmus* NII 0830, *Bacillus sphaericus* NII 0838 and *Pseudomonas denitrificans* [26] and also *Azotobacter chroococcum* [27]. *Bacillus pumilus* [28], *Bacillus thermoalkalophilus* [29], *Bacillus circulans* AB 16 [30] have also been grown on pretreated paddy straw. Paddy straw hydrolysate was also used as a fermentation medium for mold growth, *Aspergillus fumigatus* [31].

Sugarcane molasses act as very good carbon source for the biomass production. It act as cheap carbon and energy source [32,33]. Molasses substrate can support the growth of *Pseudomonas aeruginosa* and *Bacillus* sp. It has been used for poly-hydroxy alkanoates production using these organisms [34,35]. Molasses has been used in medium prepared to isolate microorganisms like lactic acid bacteria, yeast, *Bacillus* sp. etc [32]. Besides, being good carbon source molasses also contains minerals, organic compounds, vitamins that are important in the fermentation process [36]. 20% to 100% sugar waste i.e. molasses based cultivation medium was compared with lab medium for the production of *Rhizobium* biofertilizer. Growth of *Rhizobium trifolii* was found maximum at 10% level of molasses [6]. Molasses was also used as a growth medium for high cell mass and lactic acid production by *Lactobacillus salivarius* L29 [37]. Molasses supplemented with peptone (0.75%), yeast extract (0.5%) and $MgSO_4$ (0.25%) proved to be the most preferred carbon source for biomass production of four yeast strains i.e. *Debaryomyces hansenii* (S8), *Debaryomyces hansenii* (S100), *Candida sake* (S165), *Candida tropicalis* (S186) [38]. Rice supplemented with molasses (10g/l) and yeast extract (3g/l) generated highest conidia for mycoherbicide production using fungal pathogens *viz. Cochliobolus lunatus* and *Alternaria alternata* [39].

Bagasse is a lignocellulosic waste of sugarcane industry. Bagasse act as a good carbon source. It also supports the growth of nitrogenous biofertilizers such as *Azotobacter chroococcum.* Bagasse was found to be good nitrogen and organic matter source for *Azotobacter chroococcum* which was then used as a biofertilizer [40]. Sugarcane bagasse can be used for the bioethanol production. Saccharification and liquefaction of bagasse are performed for its utilization in fermentation process [41].

Another waste material from agricultural processing is cassava peel. Utilization of its solid fractions specially the peel part is limited due to low digestibility and toxicity from high levels of hydrocyanic acid [42]. Cassava starch was however used to provide nutrients to phosphorus solubilising bacterial biofertilizer. Cassava peel also acted as a carrier material for these biofertilizers [42]. Phosphate solubilizing fungal biofertilizer i.e. *Aspergillus niger*, was produced using 1% raw cassava starch and 3% poultry droppings as nutrients and 96% ground (0.5–1.5 mm) dried cassava peels as carrier material. It significantly ($p < .05$) improved the growth of pigeon pea in pot experiments [42].

Cheese whey is also an important substrate for the biomass production. Beside lactose, it also contains vitamins and minerals that improve the physiological activity of yeast. Whey can also be used for alcohol production. Lactose of cheese whey can be converted into lactic acid and galactose for the production of baker's yeast [43]. Cheese whey was reported to be used as a inexpensive growth medium for *Rhizobium loti* cells. It also enables the harvested cells to withstand freezing and desiccation. It was found to maintain the growth of *Rhizobium* in a manner related to that in recommended Yeast Extract Mannitol Agar medium [14]. Effluent from the baker's yeast industry also act as a good substrate for the growth of *Azotobacter chroococcum, Klebsiella spp, Azospirillum brasilense, Pseudomonas putida, Enterobacter spp* [44].

Different concentrations of dairy sludge was also tested for the growth of *Rhizobium.* 60% dairy sludge was found to be the superior cultivation medium for the growth of *Rhizobium* [45]. Poultry manure with the paper pulp and algal waste sludge was also used for the production of biomethane, biofertilizer and biodiesel [46,47].

Food waste is approximately one quarter of the total garbage in Taiwan. Microbial conversion of food waste to biofertilizer using *Brevibacillus borstelensis* SH168 was carried out [48]. Even waste water sludge is reported to support the growth of *Sinorhizobium meliloti* [49].

Ability of *Bacillus* to sporulate and *Azotobacter* to form cysts can be exploited to produce biofertilizer with long shelf life. Encystation of *Azotobacter* can be induced by adding 0.2% calcium carbonate or 0.3% butanol for 72 h [50]. Maximum number of vegetative cells are then transferred to dormant stage. *Bacillus* can be induced to sporulate. Polyhydroxy butyrate (PHB) production by *Pseudomonas fluorescens* A2a5 has been successfully reported [51]. For *Pseudomonas* cells to survive in liquid formulation for a long time, nutritional manipulation can be carried out to enhance the content of poly hydroxyl butyrate (PHB), a storage biomolecule [26]. It helps the bacteria to adjust to adverse environmental effects [4].

CONCLUSION

Biofertilizers are an important tool for sustaining agriculture. Biomass generation using semi synthetic microbiological medium increases the cost of production. Economical production of bacterial biofertilizers can be enhanced by using natural substrates like paddy straw, molasses, bagasse, cheese whey, dairy water sludge and cassava peel as a growth medium. These natural substrates are a good source of fermentable sugars and energy. Natural substrate have been reported to support the growth of various biofertilizers i.e. *Azotobacter* sp, *Bacillus* sp, *Pseudomonas* sp, *Rhizobium* sp and *Aspergillus niger* sp. Further work on optimizing process parameters using statistical approaches can be pursued.

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