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Biofertilizer from Discarded Media and Water hyacinth (Eichhornia crassipes)

Bandu Koli[1], Rupesh Shinde[2]
Willingdon College Sangli, Department of Biotechnology
Corresponding Author- Bandu Koli
Email- kolibandu2001@gmail.com
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Abstract

The experiment was totally based on environmental problems were use of Discarded media, Water Hyacinth (Eichhornia crassipes) are used to make an eco- friendly Biofertilizer. As we know that Agar which is used in Media plates is non- degrading and used widely in Biological Area, Water Hyacinth is another problem and the biggest problem to Environment in future and current issue, it totally destroys the water ecosystem and became a measure threat to the aquatic life. So, solving such big problems by seeing their environmental issues in future is Moto of this project.

So, water hyacinth (Eichhornia crassipes) from aquatic ecosystem and merged it in some amount of water with respect to hyacinth and under specific dark and light conditions are maintained and it is kept for 2 weeks and after that TPC (Total Plate Count) is calculated and the NPK of that liquid calculated and then to increase NPK ratio use of Nitrogen fixation and PSB media plates are used and which are discarded by various laboratories and colleges. To increase the NPK ratio cow urine is also used to make better Fertilizer. This project has totally focused on environmental threat things which are harming the environment and from the how we can make some useful product i.e., Biofertilizer.

Cow urine is a main prospect in Future for NPK ratio in biofertilizer. Some of Precipitation is formed when Water hyacinth is submerged in water after a week this precipitation is used as Manure.

Key Words: NPK, Water Hyacinth, Media Plates, Cow Urine, etc

Introduction

The commercial history of biofertilizer dates back to 1895 using "Nitrogen" by Nobbe and Hiltner (2004 Paper) with laboratory culture of rhizobium sp. When the world gets more interest in it then it simply defined by its work as a substance which contain living microorganism which, when applied to seeds, plant surfaces or soil, colonize the rhizosphere or the interior of plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (19 centaury). The microorganism in biofertilizer restores the soils natural nutrient cycle and build soil organic matter. Biofertilizer can be expected to reduce the use of synthetic fertilizers and pesticides, but they are not yet able to replace their use. Since they play several roles, a preferred scientific term for such beneficial bacteria is "Plant Growth Promoting Rhizobacteria" (PGPR).

Need-Biofertilizer such has Rhizobium, Aspergillum, Azotobacter and Blue Green algae have been used for long where they provide "eco-friendly" organic Agro- input, compare to chemical fertilizers which are at alarming stage has they became an environmental threat (Kannaiyan, 2002). Importance- by the mean of environment as agriculture is an only way to make food and fulfill the need led to use of chemical fertilizer for fast and vast yield this take to the destruction of environment and needed to solve it.

This biofertilizer is concerned on discarded media plates, Eichhornia crassipes which are threat to environment and from this waste the making of biofertilizer is there. Microbial-based fertilizer is a vital part of sustainable agricultural practice (Bloemberg et.al 2000) Haneef (et al (2014) investigated the effect of biofertilizer on cadmium-contaminated soil. They found that

Cd stress affects the growth of plants, but when they added biofertilizer to the soil, plants could better tolerate Cd. They found increased pigment concentrations when the plants were under Cd stress conditions. Similarly, (Ahmad et al. 2015) reported that Cd is a major concern in soil due to its toxic effects. They also used biofertilizer for improving the growth of plants and for increasing Cd tolerance. In addition, they found increased concentrations of proline and Phenolic compounds when treated with biofertilizers. Biofertilizer plays an important role. As Per Data that Water hyacinth is an environmental threat it is simply defined as Water hyacinth (Eichhornia crassipes) is considered to the world's worst aquatic weed species due to its invasive potential, the negative impact it has on aquatic ecosystems.

The species has been classified as a category 1b invasive species in the new 2020 Alien and Invasive Species List as part of the (National Environmental Management: Biodiversity Act, 2004 9 Act no. 10 of 2004.). The roots of water hyacinth are distinctive and hang below water surface, whereby they have a feathery appearance. Despite the harmful effects of water hyacinth on ecosystems, the plant has very attractive flowers (Villamagna MurPhy, 2010). This is because water hyacinth has a negative effect on submersed plants. Water hyacinth also interferes with immersed plant communities through crushing and pushing them. By so doing, the general ecosystem is impacted. Animal communities are negatively affected through the elimination of plants as well as blocking the access of water which animals rely on for nesting and shelter (Mariana et al, 2006). Azotobacter spp. (Gram-negative prokaryote) are considered to improve the plant health. Various mechanisms are implicated behind improved plant health in Azotobacter spp. inoculated plants (2020 Published by Elsevier B.V. on behalf of King Saud University). As we know that Microbial media plates contain Agar is non-degrading so at least its proper management should be there so Mannitol and PSB media has been taken for making biofertilizer were discarded microbial plates are used i.e.- Microbes which are in Stationary Phase are used. As we know that Nitrogen is more important for the plant were the mechanism of N2 Fixation is as below,

Media creates an Artificial environment which help microbes to grow. The media is a source of nutrients to support the growth - organisms in-vitro. The media helps in the growth and counting of microbial cells, selection of microorganisms, and survival of microorganisms. The culture medium can be liquid or gel. Bacteria are prokaryotic organisms that most commonly replicate by the asexual process of binary fission. These microbes reproduce rapidly at an exponential rate under favourable conditions. When grown in culture, a predictable pattern of growth in a bacterial population occurs. This pattern can be graphically represented as the number of living cells in a population over time and is

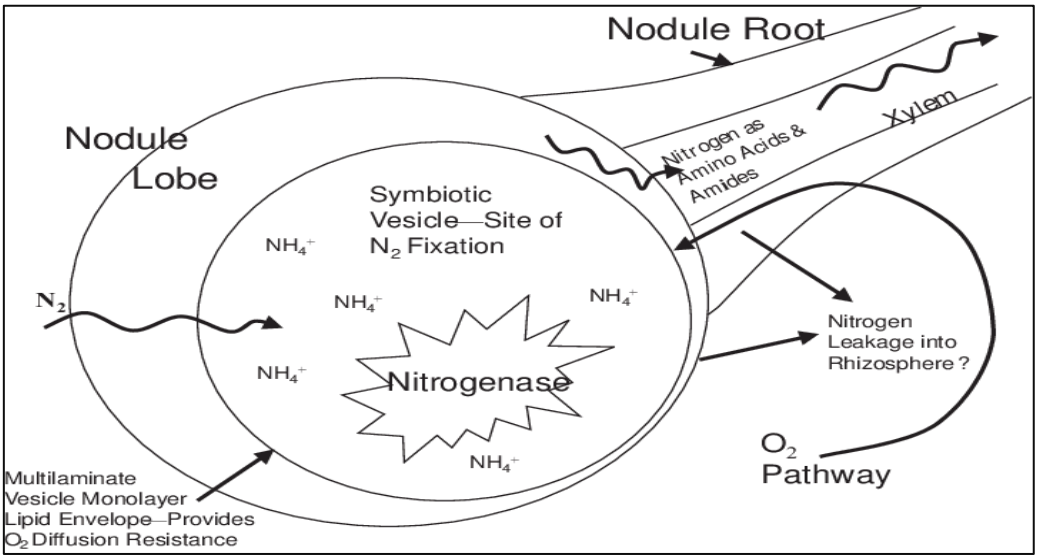

Fig 1. N2 Fixation Mechanism in Plant

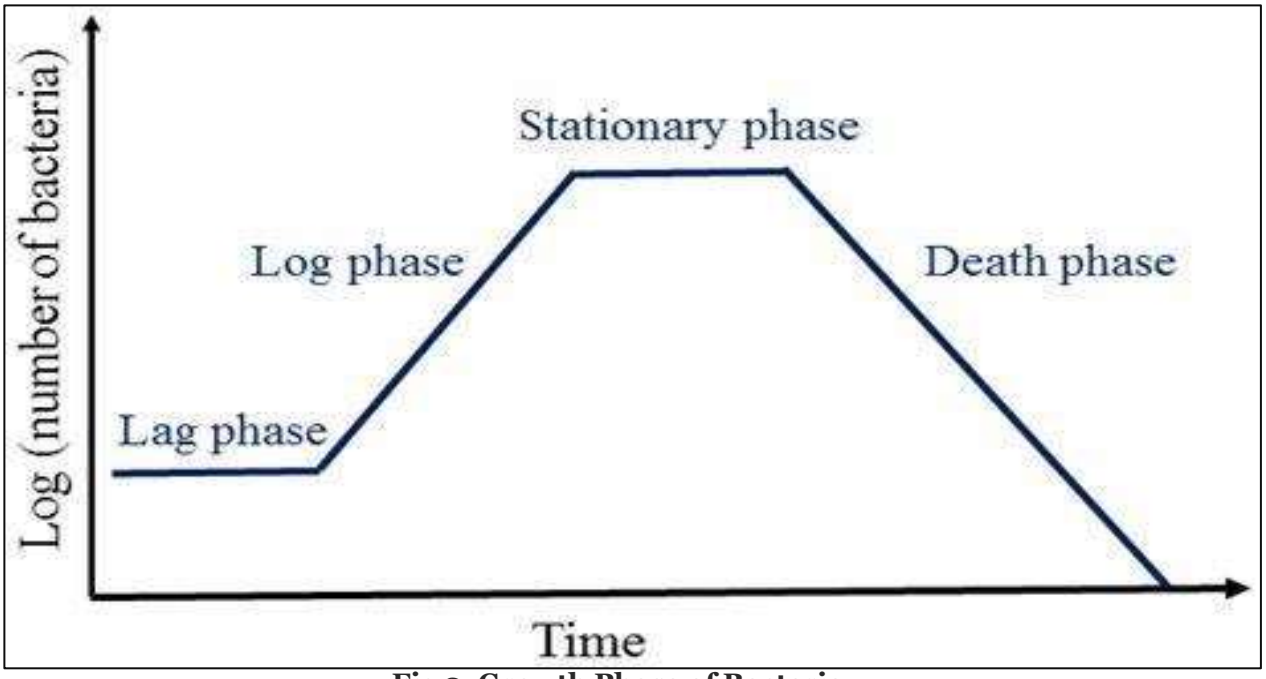

Fig 2. Growth Phase of Bacteria

known as a bacterial growth curve. Bacterial growth cycles in a growth curve consist of four Phases: lag, exponential (log), stationary, and death (Bailey, 2018).

Bacteria require certain conditions for growth, and these conditions are not the same for all bacteria. Factors such as oxygen, The bacterial growth curve represents the number of live cells in a bacterial population over a period of time. Lag Phase: This initial Phase is characterized by cellular activity but not growth. These cells increase in size, but no cell division occurs in the Phase.

Exponential (Log) Phase: After the lag Phase, bacterial cells enter the exponential or log Phase. This is the time when the cells are dividing by binary fission and doubling in numbers after each generation time. Metabolic activity is high as DNA, RNA, cell wall components, and other substances necessary for growth are generated for division.

Stationary Phase: population growth experienced in the log Phase begins to decline as the available nutrients become depleted and waste products start to accumulate. Bacterial cell growth reaches a plateau, or stationary Phase, where the number of dividing cells equal the number of dying cells. This results in no overall population growth. Under the less favourable conditions, competition nutrients increases and the cells become less metabolically active. Spore forming bacteria produce endospores in this Phase and pathogenic bacteria begin to generate substances (virulence factors) that help them survive harsh conditions and consequently cause disease.

Ph, temperature, and light influence microbial growth. Additional factors include osmotic pressure, atmospheric pressure, and moisture availability. Bacterial population's generation time, or time it takes for a population to double,

Death Phase: As nutrients become less available and waste products increase, the number of dying cells continues to rise. In the death Phase, the number of living cells decreases exponentially and population growth experiences a sharp decline. As dying cells lyse or break open, they spill their contents into the environment making these nutrients available to other bacteria. This helps spore producing bacteria to survive long enough for spore production. So, by seeing the growth curve conclusion is made that Discarded media plates are made then if they are freshly made then this Discarded media are in Phase of Stationary which is a spore Phase were microbes are in spore form. When the proper enriched condition is there then they start to grow. i.e., we are taking this discarded media and making use of that.

Materials:

Chemicals:

Ammonia, Urea (Cow manure/ Excretory part)

Detergents (Inorganic chemical for washing)

Instruments:

Laminar Air Flow

Incubator

Refrigerator

HPLC

Measuring Glass Vessels (Measuring Cylinders, Volumetric Flask)

Glassware's (Petri Dishes, Test tubes, Beaker, Flask, Spreader)

Micropipettes

Cork Borer.

Colony Counter

Microscope

Colorimeter

Sample:

Water Hyacinth

Filtrates and precipitated material (Eichhornia crassipes)

Cows Urine and Manure

Methodology

Water hyacinth is a dangerous weed for the environment as it stops the transportation of fishes and water hyacinth also absorbs the nutrients (NPK) from the water bodies and due to which there is unfavourable condition for the other plants to grow and multiply so removal of water hyacinth is important.

(A) Collection and Separation of Water Hyacinth:

Water hyacinth is collected from near lake and farm and treated with the tap water for the removal of Mud. After removal of Mud water hyacinth is cleaned and chopping of water hyacinth is done were leaves and steam part is cutted and root part is dissolved in water and leaves and steam are also dissolved in that water.

Water Hyacinth

Water Hyacinth

Water hyacinth an environmental weed by size and divided into two types Small and Big Water hyacinth where small water hyacinth is mainly grown in lakes, ponds and farms as nutrients are lesser in amount so, that much growth is not there in water hyacinth so they are in smaller in size. Big sized water hyacinth is mainly found in Sewage water, River, etc. as we know that Nutrients are in much more amount so growth is more and this water hyacinth is used as biofertilizer as this have some amount of nutrients in it and this can be helpful for the growth of plants, and by removal of this plants can also remove stress on environment Proper separation Further process after separation, water hyacinth parts are totally dissolved where the ratio is given.

Total Weight of Water Hyacinth	In 16 liters of Water
2-2.5 kg	

(B) Checking and Analysis

After two days of incubation water hyacinth containing proper analysis of Microbes and analysis of water were Alkalinity, Ph, EC, TPC, etc. Water taken for making the biofertilizer may be taken from that place were water hyacinth is grown before it is taken analysis of water is there

whether coliphage is there then the treatment of water is done by autoclave or tap water by checking it, after checking the coliphage and proper treatment of water which is used for making biofertilizer Alkalinity, PH, EC, TPC is done as a Blank value for the further making of Biofertilizer.

(C) PH, TPC, EC

After knowing the Blank value taken from the first test is taken as the first blank value for the next test growth were the PH and EC value are checked for 2 times per day to see the NPK value (whether it is increased or decreased) so proper checking of water is done. After 4-5 days TPC (Total Plate Count) is done.

(D) Use of Incubation

For the growth of N2 fixing and Phosphates Solubilizing Bacteria (PSB) Normal temperature is necessary but at 37^0 C temperature can be effective to increase the growth so use of incubator is there to enhance the growth of microbes more effectively.

E) Checking of Coliphage

After the start of making fertilizer there should be checking the Presence of coliphage So streaking or spreading of biofertilizer is done time to time on Nutrient Agar or MacConkey media.

(F) Checking of Optical Density

After the Deposition of Water Hyacinth Optical Density is checked with the help of Colorimeter.

(G) By product

After some days formation of organic matter is formed which is on the top of biofertilizer and it can be removed and used as Manure for the soil.

(H) Use of discarded media

In this use of two types of media is used, media of Nitrogen fixation media and Phosphates solubilizing bacteria

PSB Media (Pikovskays Media) Glucose (1%), Tri- calcium Phosphates (0.5%), KCL (0.02%), MgSO4.7H2O (0.19%), (NH4)2 SO4 (0.5%), MnSO4(Trace),
FeSO4 (Trace), Yeast (0.05%), Agar (1.5%), D/W (100 ml).

Mannitol N2 Free Media Mannitol (1%), MnSO4 (0.003), K2HPO4 (0.2%), NaCl (0.2%), CaCO3 (0.5%) D/W (100ml), Agar (2.5%).

The discarded media which is mainly in stationary Phase is used to enhance the microbial growth in biofertilizer.

16-20 media plates of Mannitol/PSB(Discarded)	In 16 Litters of Biofertilizer

And Further testing of Microbes is done by again TPC and NPK is calculated by HPLC Result EC and ph. Value Analysis We have taken two types of water hyacinth where One is small and grown in farmland and another is Big which is grown in Lake. The result of or Gas Chromatography. And results are analyzed.

EC & PH Value of biofertilizer made from small and big water hyacinth is given below,Small Water Hyacinth

1st Week

Tap Water

EC- 1.432

PH-7.68

Tap Water + Water Hyacinth (Small Water Hyacinth)

EC-2.500

PH-6.94

2nd Week

Tap Water

EC-1.389

PH-7.66

Tap water + Water Hyacinth (Small Water Hyacinth)

EC-3.66

PH-7.00

3rd Week

Tap Water

EC-1.3	PH-6.90

Tap Water + Water Hyacinth (Small Water Hyacinth)

EC-4.55	PH-6.88

4th WEEK

Tap Water

EC-1.00	PH-6.98

Tap Water + Water Hyacinth (Small Water Hyacinth)

EC-4.55	PH-6.88

Now analysis of big water Hyacinth also done,
Big Water Hyacinth

1st Week
Tap Water

EC- 1.432

PH-6.80

Tap Water + Water Hyacinth (Big Water Hyacinth)

EC-3.54	PH-7.12

2nd Week

Tap Water

EC-1.389

PH-7.66
Tap Water + Water Hyacinth (Big Water Hyacinth)

EC-4.80	PH-6.80

3rd Week

Tap Water

EC-1.3

PH-6.90

Tap Water + Water Hyacinth (Small Water Hyacinth)

EC-5.55	PH-6.88

4th WEEK

Tap Water

EC-1.23

PH-6.98

Tap Water + Water Hyacinth (Small Water Hyacinth)

EC-6.66	PH-7.10

Result of Laboratory analysis of NPK values,

Sr.No.	Parameters	ppm
1	Nitrogen	70.52
2	Phosphorous (as P2O5)	29.42
3	Potassium (K2O)	199.05
	First Result	

Sr.No.	Parameters	ppm
1	Nitrogen	95.27
2	Phosphorous (as P2O5)	8.43
3	Potassium (K2O)	220
	Second Result	

After the result which was carried out on ICP (Inductive Coupled Plasma) we get to know that addition of Water hyacinth and discarded media there is increase in the ratio of NPK and more the amount on Water hyacinth and discarded media then the result would be in percentage.

Discussion

Biofertilisers are products that contain microorganisms essential for soil fertility and plant growth when added to the soil. A biofertilizer is a chemical that contains living microorganisms that colonize the rhizosphere or the interior of the plant when given to seeds, plant surfaces, or soil, and encourage growth by controlling the quantity or availability of primary nutrients to the plant host. Biofertilisers supply nutrients to plants through natural processes such as nitrogen fixation, phosphorus solubilization, and the creation of growth-promoting chemicals. They help restore the soil's natural nutrient cycle and increase soil organic matter.

By seeing the ph and EC values in project there is increase in the ph and EC values as we see the blank values when the Water hyacinth is not mixed with the Tap water then the EC and ph values are steady but when water hyacinth is there then after weeks there is increase in ph and EC values. We have seen that ph and EC value of before introduction of small water hyacinth into the

Tap water -

EC- 1.432
PH-7.68
After introducing Eichhornia crassipes into the Tap water,

EC-2.500	PH-6.94

As there is increase in EC and ph value because ions are disposed by Eichhornia crassipes into the water and so ph is also varying. After four weeks ph and EC of tap water there is steady ness in tap water

EC-1.00

PH-6.98
After four weeks analysis is stopped as ph and EC value is not varying and there is start of declining phase of ph and EC.

EC-4.55

PH-6.88
Now big water hyacinth is also analysed and compare to small water hyacinth it gives out more ions so there is more ph and EC value
Comparing the Tap water before mixing the big water hyacinth,
EC- 1.432

PH-6.80
After introduction of water hyacinth,
Tap Water + Water Hyacinth (Big Water Hyacinth)
EC-3.54

PH-7.12
After four weeks there is steadiness in tap water and main samples in ph and EC values
4th WEEK
Tap Water
EC-1.23

PH-6.98
Tap Water + Water Hyacinth (Small Water Hyacinth)

EC-6.66

PH-7.10

After seeing and analysis we get to know that there is increase in the ph and EC values after introduction of water hyacinth which is also influenced in NPK values. And after introduction of microbes when are isolated from this fertilizer specific known and essential microbes are found when colony count is performed good growth of microbes are seen. NPK ratio should be increased from ppm to some percentage so more the Concentration of water hyacinth is needed. Proper analysis of microbes seeing

contamination and increasing the concentration of NPK can make the biofertilizer more proper and to be good for the crop yield.

Conclusion

The described part of Upstream Process for Production of biofertilizer with the help of Water Hyacinth and Discarded Media. Were there is increase in NPK ratio when water hyacinth for making Biofertilizer where Alkalinity is increased as we get to know that Roots of water hyacinth releases its nutrient into the water or we can also use the which is from the original path where we take water hyacinth for better result.When discarded media of PSB/Mannitol of microbe's Phosphates solubilizing bacteria and Azotobacterial for Nitrogen Fixation and get to know that when they are in stationary Phase the form spores when we inoculate them in biofertilizer as the proper environment make them sustainable to grow and we get that their growth is increased in biofertilizer. After NPK ratio analysis there should be more percentage of water hyacinth to give more concentration percentage of NPK.

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