"Education for Knowledge, Science and Culture"

-Shikshanmaharashi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

Vivekanand College, Kolhapur (An Empowered Autonomous Institute)

Department of Biotechnology Optional

10/01/2025

NOTICE

Hereby it is informed to all students of B.Sc. III, your open book test of Environmental Biotechnology will be arranged, at 12.15 pm, Friday 18/01/2025. It is compulsory all to attain it. Topic for open book test is **Azatobacter as Bio fertilizer**

VIVEKANAND COLLEGE ESTD. JUNE 1964 KOLHAPUR-416 003

Head of Department

HEAD
DEPARTMENT OF BIOTECHNOLOGY (OPTIONAL)
VIVEKANAND COLLEGE, KOLHAPUR
(EMPOWERED AUTONOMOUS)

"Education for Knowledge, Science and Culture"

-Shikshanmaharashi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

Vivekanand College, Kolhapur (An Empowered Autonomous Institute)

Department of Biotechnology Optional

B.Sc. III 2024-25

Roll no.	Name of student	Signature
8360	ATTAR SANIYA SALIM	
8361	AWATE GAYATRI ANIL	
8362	BAGWAN HEENA SHAFIK	
8363	DESAI SUSHANT KADAPPA	
8365	EKSHINGE VRUSHALI SHIVAJI	
8367	KARUNAKAR MUDITA S.	
8369	KAZI SADIYA ZAKIRHUSEN	
8370	KUCHEKAR SWAPNIL BAPU	
8371	KUMBHAR DIKSHA R.	
8372	KUNDALE SHRUTI MILIND	
8373	LAVHATE SNEHA MARUTI	
8374	LOHAR SAYALI PUNDLIK	
8376	MANDEKAR RUTUJA M	
8378	PATIL NEETA BALIRAM	
8378	PATIL RUTUJA SARDAR	
8379	PATIL SHREYA BAJIRAO	
8380	PATIL SNIGDHA SUNIL	
8381	SHEWALE YOGESH SUJIT	
8382	SONAWANE NIHARIKA DEEPAK	
8383	SURYAWANSHI ADITI VINOD	
8384	UNHALE PRIYANKA SHIVAJI	
8385	KRITIKA BHARAT VORA	
8386	WADHWA MAHEK VINOD	
8366	KAMBALE SANIKA SANJAY	
8368	KASHID DHANSHRI UTTAM	
8364	DONGARE SONAM BALASO	
8375	MAHADIK SAKSHI SADASHIV	

"Education for Knowledge, Science and Culture"

-Shikshanmaharashi Dr. Bapuji Salunkhe

Shri Swami Vivekanand Shikshan Sanstha's

Vivekanand College, Kolhapur (An Empowered Autonomous Institute)

Department of Biotechnology Optional

B.Sc. III 2024-25

Roll no.	Name of student	Mark
8360	ATTAR SANIYA SALIM	15
8361	AWATE GAYATRI ANIL	18
8362	BAGWAN HEENA SHAFIK	19
8363	DESAI SUSHANT KADAPPA	12
8365	EKSHINGE VRUSHALI SHIVAJI	14
8367	KARUNAKAR MUDITA S.	13
8369	KAZI SADIYA ZAKIRHUSEN	20
8370	KUCHEKAR SWAPNIL BAPU	15
8371	KUMBHAR DIKSHA R.	10
8372	KUNDALE SHRUTI MILIND	12
8373	LAVHATE SNEHA MARUTI	13
8374	LOHAR SAYALI PUNDLIK	14
8376	MANDEKAR RUTUJA M	13
8378	PATIL NEETA BALIRAM	18
8378	PATIL RUTUJA SARDAR	10
8379	PATIL SHREYA BAJIRAO	14
8380	PATIL SNIGDHA SUNIL	16
8381	SHEWALE YOGESH SUJIT	14
8382	SONAWANE NIHARIKA DEEPAK	15
8383	SURYAWANSHI ADITI VINOD	17
8384	UNHALE PRIYANKA SHIVAJI	12
8385	KRITIKA BHARAT VORA	15
8386	WADHWA MAHEK VINOD	16
8366	KAMBALE SANIKA SANJAY	10
8368	KASHID DHANSHRI UTTAM	10
8364	DONGARE SONAM BALASO	13
8375	MAHADIK SAKSHI SADASHIV	15

Subject Teacher.

॥ ज्ञान, विज्ञान आणि सुसंस्कार यासाठी शिक्षण प्रसार ॥
- शिक्षणमहर्षी डॉ. बापूजी साळुंखे
(अधिकारप्रदत्त स्वायत्त)
कोल्हापूर
VIVEKANAND COLLEGE, KOLHAPUR.
(Empowered Autonomous)

18/20

SUPPLIMENT

Gayatri Anil Awate.

Jr. Supervisor's Sign.	
Students Sign. :	Gawate
Seat No. :	8361
Seat No. in words :	
Suppliment No. :	40335
Centre	

प्र. क्र. Q. No.

1) Azatobacter inoculant :-

① Azatobacter is free-living, ~~non-symbiotic~~, Heterotrophic, non-symbiotic nitrogen fixing & pleomorphic organism.

② The Azatobacter ~~species~~ produce structure called cyst in adverse condition.

③ The Azatobacter species such as
i) A. chrococcum
ii) A. insignis.
iii) A. agilis.
iv) A. beijerinkii

④ Azatobacter is grown on nitrogen free mannitol agar medium & Jensen's medium.

⑤ They produce soft, milky & mucoid colonies.

⑥ The Azatobacter isolation is done streak or spread plate technique.

⑦ The optimum temp^r is 25-30°c & optimum pH is 7.2-7.5.

02 Section | Q. No. | Marks

प्र. क्र.
Q. No.

Isolation of Azatobacter -

① The isolation of Azatobacter can ~~is~~ isolated by spread or streak plate technique.

② This is the most advance method.

③ The isolation of Azatobacter is done on nitrogen free mannitol agar medium or Jensen's medium.

④ The material used for the isolation of Azatobacter is g/l

i) KH_2PO_4 - 0.5
ii) $MgSO_4 \cdot 7H_2O$ - 0.1
iii) NaCl - 0.1
iv) D/W - 1000 ml
v)

⑤ The Azatobacter is isolated from soil & make suitable serial dilution of soil sample 10^5 - ~~10^6~~/ml & spred or streak the on the ~~on~~ N_2 free mannitol agar.

⑥ Also the soil lumps are used for isolation of Azatobacter.

⑦ The Azatobacter is grow ~~after~~ in 30°C for 3 days.

Section | Q. No. | Marks | 03

Q. No.

Mass Production :-

① The Azatobacter is produce on N_2 free mannitol agar medium.

② The production of Azatobacter is require some suitable conditions such as Aeration, Agitation. pH. Temperature. etc.

③ The Azatobacter species present 10^5/ml.

④ The Azatobacter require optimum temp° 30°c & pH - 7.2.

⑤ The Azatobacter grow after 96hrs.

⑥ The sterilization is maintained in fermentor.

⑦ The Azatobacter mass production helps in agriculture for Nitrogen fixation.

⑧ The black brown pigment is due to the mannitol oxidation of mannitol by tyrosinase.

⑨ The Azatobacter produce some substances such as IAA, GA, vitB & Antifungal substances.

04 Section Q. No. Marks

प्र. क्र. Q. No.

* starter culture, curing & packing - (carrier).

① The culture of Azatobacter speci is grow on N₂ free mannitol agar medium or Jensen's medium.
② In the starter culture the mass production should be used.
③ The culture will be packed in polythene bags.
④ Mention the name of biofertilizer, recommended crop, batch number, Manufacturer address etc mention on the polythen bag.
⑤ The packed bags are stored at 4°c - 15°c in cold condition.
⑥ legnite, pit soil are used as carrier.

A methods of Application :-

① seed treatment :-

→ The seed treatment is done with the starter culture. as following -

10g Jaggery + 400ml water boil & mix & cool.

↓

Add the mixture of Jaggery & water in starter culture.

↓

Pour on seed & spread on ground.

॥ ज्ञान, विज्ञान आणि सुसंस्कार यासाठी शिक्षण प्रसार ॥
- शिक्षणमहर्षी डॉ. बापूजी साळुंखे

(अधिकारप्रदत्त स्वायत्त)
कोल्हापूर

VIVEKANAND COLLEGE, KOLHAPUR.
(Empowered Autonomous)

SUPPLIMENT

Jr. Supervisor's Sign. :	
Students Sign. :	
Seat No. :	
Seat No. in words :	
Suppliment No. :	40336
Centre	

प्र. क्र. Q. No.

② seedling treatment -
The seedling treatment on seed is advance method & useful in agriculture.

③ Foliar Application -

④

07

02 Section	Q. No.												
	Marks												

प्र. क्र.
Q. No.

② Nodulation Test -

① The nodulation test is for rhizobial inoculant.

② Nodulation is present in plants roots

③ ~~plant roots~~ Nodulation is present in leguminous plant
eg - Pea, Groundnut etc.

④ These leguminous plants fixes Nitrogen with the help of Nodules

⑤ The organism present in nodules ~~for~~ are known as Rhizobium.

⑥ This method is advance method.

⑦ The organisms of legumenious plant are gram negative.

⑧ The ~~nodulation~~ nodules are present on root at the ground.

⑨

04

Section | Q. No. | Marks

प्र. क्र. / Q. No.

③ Vermicompost -

① Vermicompost is process of biotechnology that compost kitchen waste, agricultural waste etc.

② the worms are rarely found in ~~good~~ that is Red wigglers & worms.

③ vermicompost is very useful compost in potting plant & in agricultural crop.

④ vermicompost is rich in nutrient & minerals.

⑤ Vermicompost is ~~or~~ made by occures by two ways.
① Bed method.
② Pit method.

⑥ vermicompost is highly enriched for plant growth.

⑦ vermicompost is high ammonia content.

⑧ The addition of carbon the decomposition of waste is reduces.

⑨ the worms compost the waste. & the produce vermicompost.

⑩ The duration for this process is 45-50 days.

⑪ The vermicompost requidy shedy, moist, & small bound. ~~or~~ area. for decomposition of waste.

04

⑫ cow dung also used for vermicomposting.

Vrushali. S. ekshinge

॥ ज्ञान, विज्ञान आणि सुसंस्कार यासाठी शिक्षण प्रसार ॥
- शिक्षणमहर्षी डॉ. बापूजी साळुंखे

(अधिकारप्रदत्त स्वायत्त)
कोल्हापूर

VIVEKANAND COLLEGE, KOLHAPUR.
(Empowered Autonomous)

14/20

SUPPLIMENT

Jr. Supervisor's Sign. :	Wajpai
Students Sign. :	Vrushali
Seat No. :	8365
Seat No. in words :	
Suppliment No. :	40338
Centre	

प्र. क्र.
Q. No.

1)

Azotobactor Inoculant -

Introduction-

i) Azotobactor is heteromorphic, free living, non-symbiotic, N_2 fixing, Gram negative motile organism.

ii) It produces resistant cyst in adverse conditions.

iii) It produces growth promoting hormones like Indole acetic acid, Gibberlins, vit B & antifungal substances.

iv) Azotobactor can be isolated on N_2 free Manitol medium or jensen's medium.

v) Azotobactor forms dark brown melanin pigmentation. ~~meta~~ due to melanin oxidised by tyrosinase.

vi)

02 Section | Q. No. | Marks

प्र. क्र. Q. No.

Isolation :-

i) Azotobactor species can isolated by soil dilution method.

ii) In this method 0.1 ml of suitable dilution is either streaked or spread on the N_2 free Mannitol medium.

iii) Then the plates are incubated at 30°c for 3 days.

iv) after 3 days azotobactor produces soft, milky, mucoid colonies.

v) Azotobactor can also isolated by direct spread soil clumps on the N_2 free medium.

vi) The medium used for this is usually the Jensen's medium.

vii) The composition of Jensen's medium is sucrose 20, KH_2PO_4 - 1.0, $MeSO_4 \cdot 7H_2O$ - 0.5, NaCl - 0.5 water - 1000 ml & pH is self adjusted.

viii) The colony of Azotobactor is transfered to inoculant in 250ml capacity flask.

ix) Then it is incubated on rotary Shaker at 30°c for 3 days.

x) This is called 'starter culture'.

xi) This is used to charging the large fermentors.

- Mass production :-

i) Mass production of Azotobactor can done either in large flasks or in batch fermentors.

ii) The fermentor is most automatic & accurate for multiplication of any organism.
iii) In this method the medium is added to fermentor & sterilize it. The pH is adjested to 7- 7.2.
iv) The various parameters are adjusted during fermentation like aration, agitation, temp-24°c & pH- 7- 7.2.
v) The ~~the~~ inoculant is added to the ~~medium~~ carriee which is sterilized early.
vi) It is incubated for 10- 12 hrs.
vii) Aftee this when the cell density reaches to 5×10^9 cells/ml the culture is given out from fermentoe.

- Carriee, curing, packaging & storage.

- Carriee- mostly lignite, compost, pit soil used as carriee for azotobactoe isolation.
- The lignite is in large flaks so it is grinded into powdee by grinding machine.

- Curing :-
The inoculants & sterilized carriee mixed properly & the organisms utilize the remaining constituents & medium also accilimatize itself. The process is knocon as 'Stabiliz-ation' or 'curing'.

04 Section | Q. No. | Marks

प्र. क्र.
Q. No.

- Packaging & storage -
The inoculant is filled in the 250g - 500g capacity Polythene bags.
- The bags are sealed by leaving one-third space by electric sealer.
- Then the bags are simultaneouly proceed to labeled with name of fertilizer, name & address of manufacturer, batch no, date of packing & expire, crops to be used, composition of material, etc.
- Then the polythene bags are stored at cool temp (4-16°c).

- Methods of Applications -
1) Seed treatment

40g jaggry disolve in 400ml water & by boiling & cool (gum arabic also added as a sticker)
↓
Mix it with inoculant properly)
(charcol inoculant is used)
↓
pour it on the seeds uniformly
↓
Dry the seeds under sun
↓
Sow or transplant the inoculant coated seeds immidiately after drying.

Vaishali. S. ekshinge (2)

॥ ज्ञान, विज्ञान आणि सुसंस्कार यासाठी शिक्षण प्रसार ॥
- शिक्षणमहर्षी डॉ. बापूजी साळुंखे

(अभिमतविद्यापीठ दर्जा)
कोल्हापूर

VIVEKANAND COLLEGE, KOLHAPUR.
(Empowered Autonomous)

SUPPLIMENT

Jr. Supervisor's Sign	
Students Sign	Vaishali.
Seat No.	8365
Seat No. in words	
Suppliment No.	40379
Centre	

प्र. क्र.
Q. No.

2) Seedling treatment

3) Top dressing :-
In this method 40g of inoculant diluted by adding water. & directly apply on the surface of soil.

a) pouring method :-
The inoculant is diluted with water to make

02 Section | Q. No. | Marks

प्र. क्र. Q. No.

slurry pour at the near to roots.

5) Soil treatment

6) foliar treatment -
The 1:10 dilution inoculant which contains 5×10^9/ml foliar is used.

07

Q2)

1) Vermicomposting -

1) Vermicomposting the process in which earthworms converts the organic waste into digested form.

2) Vermicomposting is the scientific process of forming compost by using earthworms.

3) They are mostly found in soil feeding on biomass & excreation of digested forms of substance.

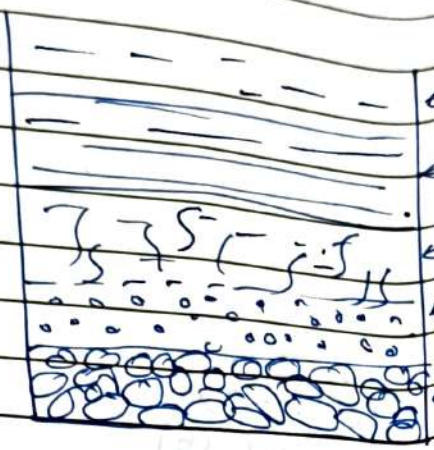
4) Vermicompost means 'worm - farming' which uses organic matter & their excreta contain large amount of nitrates & minerals like potassium, phosphorous, magnessium, calcium.

5) There are 500 sp of earthworm in India & 3000 sp. over known world.

6) It is firstly studied in canada.

Q. No.
Marks

process -



1) The process start with the making the plastic or cement pits.
2) Bio mass is collected & chopped into small pieces
3) The prepare a cowdung slurry.
4) At the bottom there is ~~leve~~ layer of pebbles or bricks.
5) Then the layer is covered with sand.
6) Then the slurry & cowdung is layered for easy degradation.
7) Then the chopped biomass & other biodegradable materials are added in a layer.
8) Then the earthworms are introduced into the pits.
9) Abore that there is layer of loamy soil or ~~dor~~ cowdung slury is applied.
10) The tank / pits are covered with lid to avoid rain water & excess Sunlight.

04

04 Section | Q. No. | Marks

प्र. क्र.
Q. No.

② Mass production of Cyanobacteria -
There are several methods for mass production of cyanobacteria.

① Pit method -
1) In this method the pits are created into the soil.
2) The pits with 2X1.0X0.2 width are formed & covered with polythene bags.
3) Then the 10 cm water is added & 2gm of inoculant is added.
4) Then other growth nutrient such as phosphate Super phosphate, lime is added.
5) then 2ml of malathione also added to avoid mosquitoes.
6) The it is incubated for 5-10 days.
7) After 5-10 days the inoculant is evaporated & dry it.
8) Then packed into polythene bags.

② Field method -
1) In this method, the loamy soil field of 120 sq/ft is used.
2) In this method the surface is puddle
3) Then it is covered by earthen bunds about 15 cm in height.

Vrushali

(2)

॥ ज्ञान, विज्ञान आणि संस्कार यासाठी शिक्षण प्रसार ॥
- शिक्षणमहर्षी डॉ. बापूजी साळुंखे

(अधिकारप्रदत्त स्वायत्त)
कोल्हापूर

VIVEKANAND COLLEGE, KOLHAPUR.
(Empowered Autonomous)

SUPPLIMENT

Jr. Supervisor's Sign. :	
Students Sign. :	Vrushali
Seat No. :	8365
Seat No. in words :	
Suppliment No. :	40380
Centre	

प्र. क्र.
Q. No.

a) Then add 10 cm water & 20 gm of inoculant
5) Then add 20 gm super phosphate, molypddte, lime & 20 ml of Malathione.
6) It is incubated at 5-7 days.
7) The full flacks are formed within 21-28 days.

03

Divya Raghunath Kumbhar

VIVEKANAND COLLEGE, KOLHAPUR.
(Empowered Autonomous)

SUPPLIMENT

Supervisor's Sign
Students Sign
Seat No 8371
Seat No in words
Supplement No 1
40302
Centre V.C.K

प्र. क्र.
Q. No.

Que 1) long Note.
1) Explain the Azotobacter inoculants :-
1) Azotobacter it is heterotrophic
2) It is free living and Nitrogen fixing bacteria.
3) Azotobacter are gram negative bacteria
4) It is motile.

- Isolation.
- Azotobacter are growth promoting bacteria.
- Included species that is A. chroeccurase
- Azotobacter grow on optimal temperature that is 25-30°C.
- It is optimal pH that is 7-7.2 pH.
- Azotobacter are N_2 fixing bacteria.
- Azotobacter are growth promoting that is IAA, ~~Gibbrel~~ GA and Vit B12.
- Azotobacter are less effective on organic soil
- Isolation done by soil dilution technique

02 Section | Q. No. | Marks

प्र. क्र.
Q. No.

- Carrier, Curing, Packing & Storage -
  1) Carrier - lignate, are mostly used material For as a Carrier also used material Mud, Soil
  2) Curing - In the Curing process Carrier are mixed with lignate at ration 1:2 mixed properly
  3) Packing - after curing process packing that in 250g to 300g polythene bages
     - living air For Space
     - leabbeled and Seal that bages
  4) Storage - After that three process last Step is Storage in Cool temperature
     - maintain at 4°C
     - optimal Cool temperature are efficient
     - Cool temperature is required
- Mass production -
  - we used large flask For Azotobacter mass production.
  - Serially Soil dilution technique used
  - Azotobacter inoculants are Spread or Streak on mannitol agar plate. after that
  - inubation at 30°c For 3 days
  - Result are the Azotobacter are produce Soft, milky & mucoid in their nature

Section | Q. No. | Marks | 03

प्र. क्र. Q. No.

2) Short Note

1) Vermicomposting:-

1)- Vermicomposting are earthworm to break down organic waste like kitchen & Scrape & yard waste into nutrient rich Compost

2) In Vermicomposting special used ~~red~~ wig, red wiggler are

3) Vermicomposting worm ~~eta~~ eats the organic material in Soil

4) another many types in Composting that means other Composting technique
- adsorption composting
- regendation Composting etc

5) The vermicomposting is a natural process here no harmful toxics & released by the process

6) The soil is spread over a surface on which ~~leavet~~ leaves layer is horizontally spread on & then the waste material i.e organic waste material are spread and again covering the surface, addition of some earthworms on the surface & they get sufficient food

04 Section | Q. No. | Marks

प्र. क्र.
Q. No.

growth this is short process in the vermicompost.

7) Avoid plastic., surgical mask and other

8) Vermicomposting process in all over depend on natural waste materials.

9) used material leaves, fruits,

10) very used & beneficial plant growth

03

Disha Raghunath Kumbhar

VIVEKANAND COLLEGE, KOLHAPUR.
(Empowered Autonomous)

SUPPLIMENT

Jr. Supervisor's Sign
Students Sign
Seat No. 8371
Seat No. in words
Suppliment No. 2
40303
Centre V.C.K

Q. No.

2) Phytoremediation

1) The process of degradation of Contamination in the soil by using plants are called phytoremediation

2) They are used to immobilised the toxic Contamination

3) It helps the soil to toxic free

4) the toxic free soil help to generate new & healthy plant growth

5) There are typed -
   1) Phytoextraction
   2) Phytodegradation
   3) Phytotransformation
   4) Phytovolatile
   5) Rhizofertilizer.

1) Phytoextraction -
It is used to extract the bacteria from the soil to shoot. it enters from the root nodules to shoot & leavel of the plant

02 Section | Q. No. | Marks

प्र. क्र.
Q. No.

2) Phytodegradation - It happens below the soil level in the water from the water is the uptapped.
3) Phytostabilizer - used to stabilize the plant chemical
4) Phytovolatile - Show volatility
5) Rhizofertilizer - introduced in meats & make soil fertile.

03