Chapter-3

Development of Enriched Microbial Consortium

3.1 Collection of floral waste

Flower wastes were collected from the following selected shrines, including Rajkot, Haridarshanam temple, events, worship, rituals, festivals, etc. We collected flower waste included marigold and rose flowers, among others. In this study, only flower waste was used to treat soil without stems, roots and leaves.

Fig. 3 Collection of floral waste from the different temples and suitable sources

3.2 Sample collection from Gaushala

For the isolation of floral waste degrading microorganism we collect following samples from Satyakam Gaushala, Rajkot, in which we have image of cow dung, cow urine and gaushala soil sample and cow saliva and skin samples image capturing process not accessible.

- Cow Dung (Indigenous Gir Cow)
- Cow Urine (Indigenous Gir Cow)
- Cow saliva (Indigenous Gir Cow)
- Cow skin (Indigenous Gir Cow)
- Gaushala Soil

Fig.4 Satyakam Gaushala, Rajkot

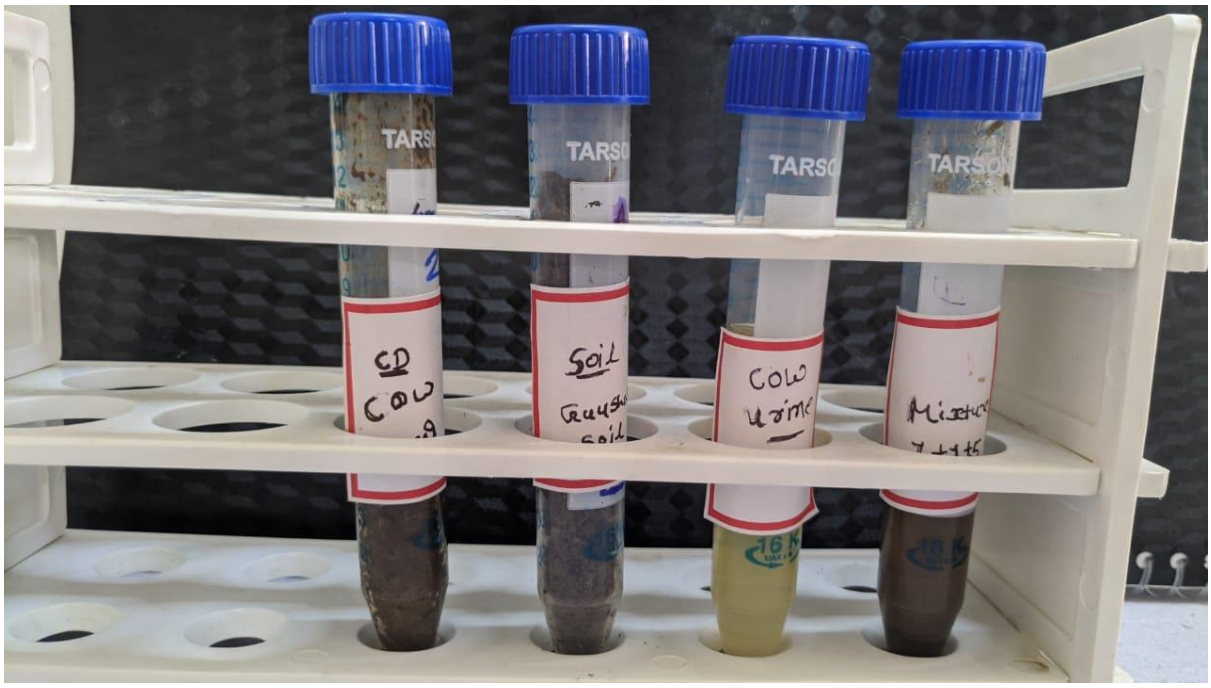

Fig. 5 Collected Samples from gaushala

3.3 Extraction of Floral Waste

After aggregation floral waste from varied locations, perishable waste containing wreaths and flowers was separated from non-biodegradable elements containing plastic, paper, twine, and different waste. The detached flower shards were detached on paper for forty-eight hours to air dry. The dry samples were then ground in an exceedingly mixer processor to supply three hundred cc of flour starch paste. The homogenized mixture was factory-made once more in an exceedingly combination processor. This mixture then let sit for 3 hours to permit any dust to settle. The textile was sieved and therefore the clear supernatant was extracted. The ensuing filtrate was delineated as floral concentration (Mulay et al., 2020.).

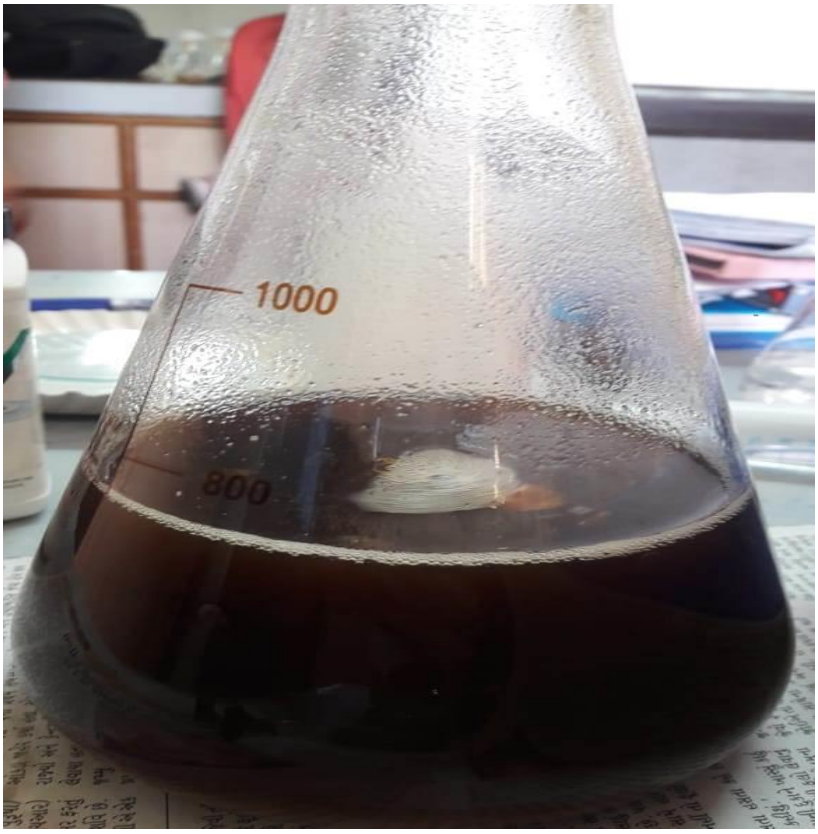

Fig.6 Extracted liquid from the floral waste

3.4 Media preparation from the floral waste

The unique pH of the floral extract was 4.7, which was too acidic to allow for the development of typical microbes. As a result, pH was adjusted to 7.2 and 5.6 to separately support the growth of microorganisms. 3.0 g/100 ml of agar powder was added to the flower extract for media hardening, which was followed by media sanitization at 15 psi for 30 minutes at 121 °C (Mulay et al., 2020.).

Fig.7 Floral waste agar plates

3.5 Isolation and Screening of floral waste degrading microbes

Samples of Indigenous Gir Cows dung, urine, saliva, skin and gaushala's soil were collected from Satyakam Gaushala. 100 cc of flower waste medium was inoculated with 1 g of each collected sample and mixture of all sample test. These vessels underwent a 3-day incubation period at 28 °C and 125 rpm. Allowing the debris to settle, leave the jar alone for two hours. The plant effluent medium was then covered with the diluted supernatant. Plates were incubated at 28°C until a deposit appeared on an agar plate to indicate progress (R et al., 2013).

3.6 Microbial Consortium Development at laboratory and Pilot Scale

Using floral waste agar plates, we are able to identify Seventeen different bacterial colonies that are capable of degrading floral waste. These bacterial isolates were used as biocompost. For the preparation of the consortium, it was decided to use the combinations that quickly decompose floral wastes. A minimum broth comprising flower wastes was used to inoculate an old bacterial culture of different life forms in specified combinations. 48 hours were spent incubating the stock at 37°C. Following incubation, this broth was used as a consortium, and 20% (v/w) of this consortium was then added to the flower wastes as inoculums (Pindi, 2012).

3.7 Results:

3.7.1 After inoculation of Indigenous Gir cow's sample on floral waste medium we have obtained diversity of bacterial and yeast strains. So, we perform Gram's staining procedure and morphological analysis for primary identification.

Fig.8.1 Microbial growth on floral waste agar plate-1

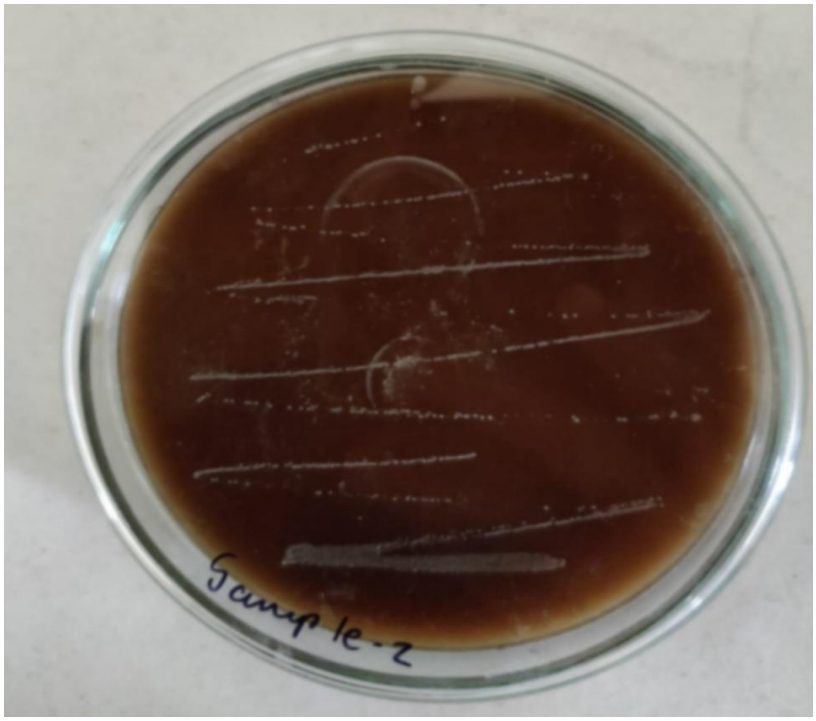

Fig.8.2 Microbial growth on floral waste agar plate-2

3.7.2 Light Microscopy Result illustrating the Gram Positive Bacteria diversity from Gir Cow & Gaushala Soil

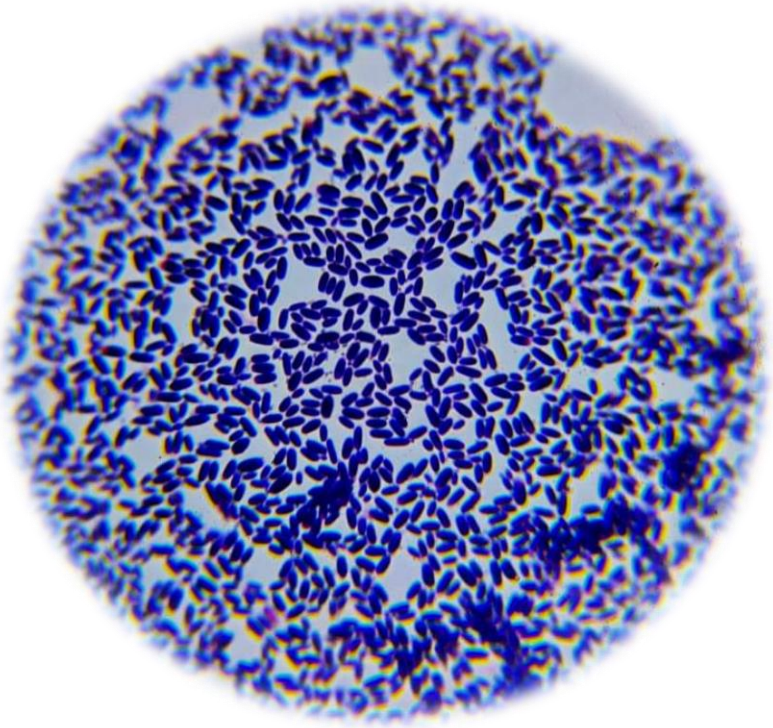

Fig 9.1 Gram Positive Rod Shaped Bacteria

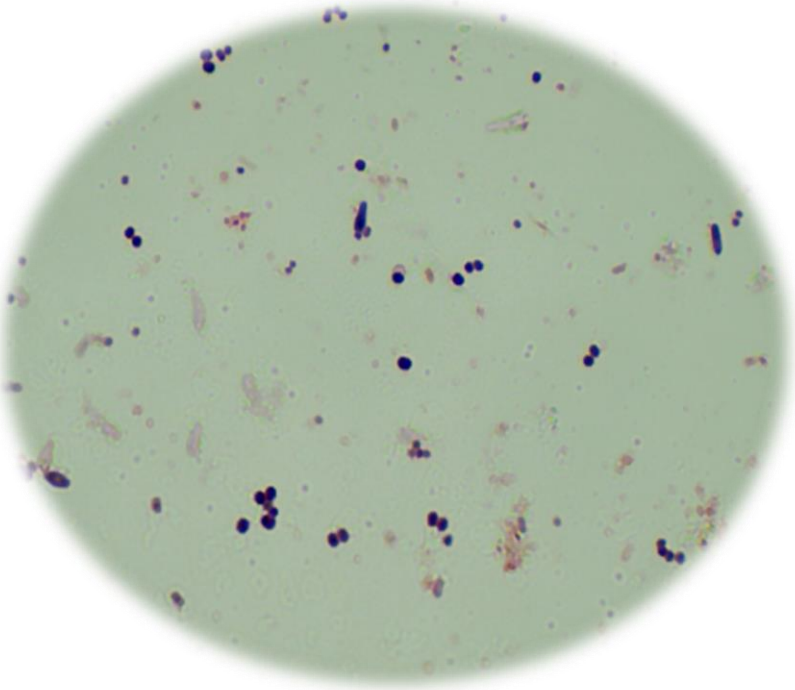

Fig 9.2 Gram Positive Cocci Shaped Bacteria

3.7.2 Light Microscopy Result illustrating the diversity of Microbial Consortium from Gir Cow & Gaushala Soil

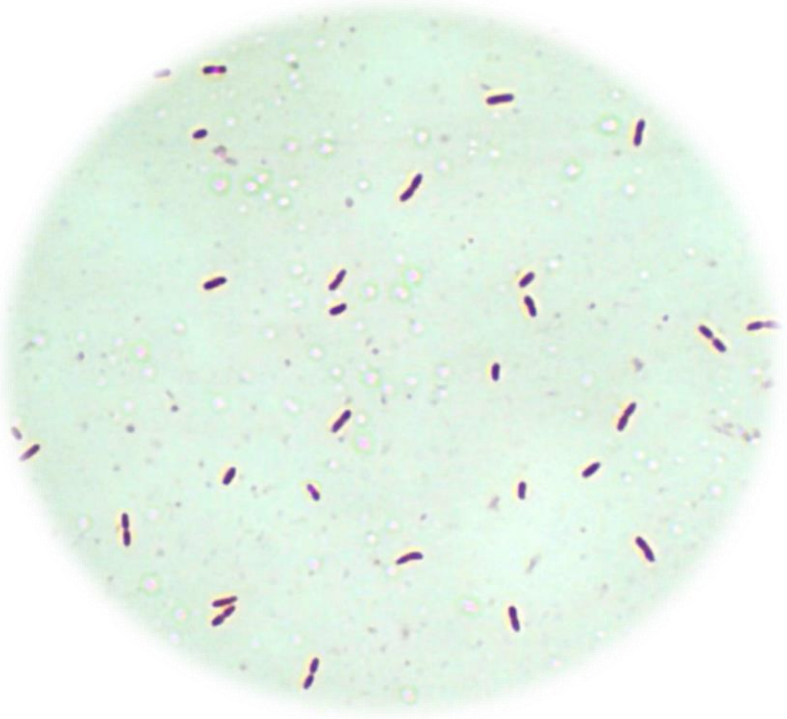

Fig 9.3 Gram-Negative Rod-Shaped Bacteria

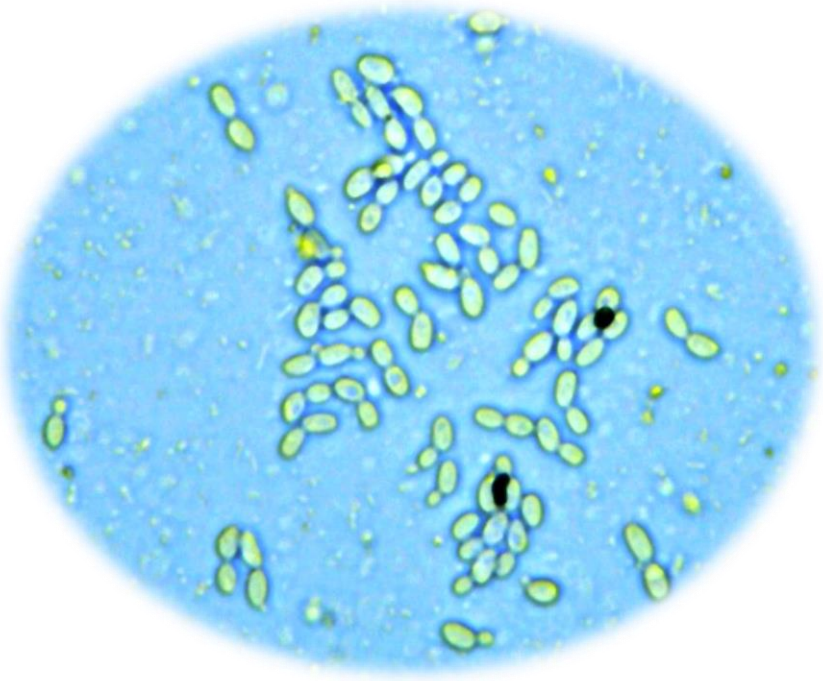

Fig 9.4 Large Oval Shaped Bacteria found in group

3.7.3 Phase Contrast Microscopy Result illustrating the diversity of Microbial Consortium from Gir Cow

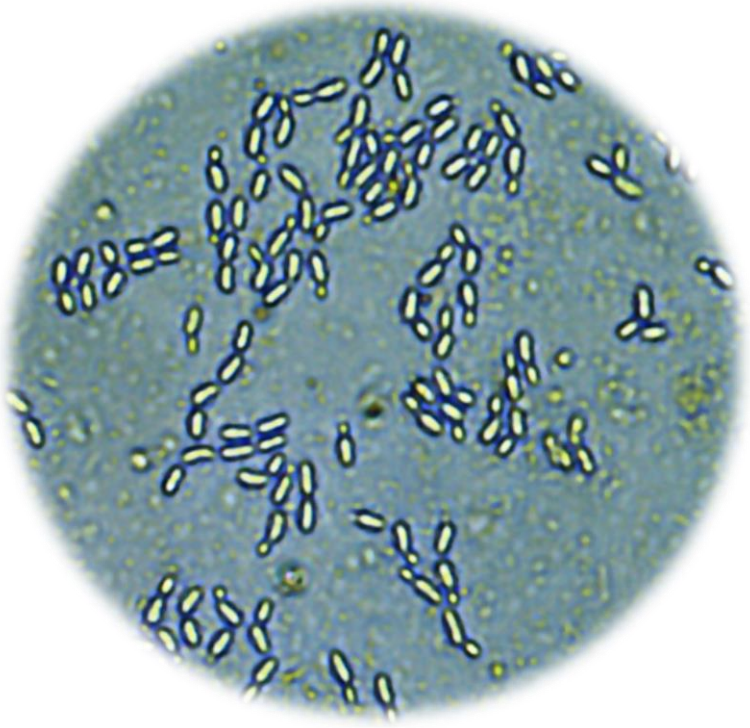

Fig 9.5 Medium Sized Oval Shaped Bacteria found in group

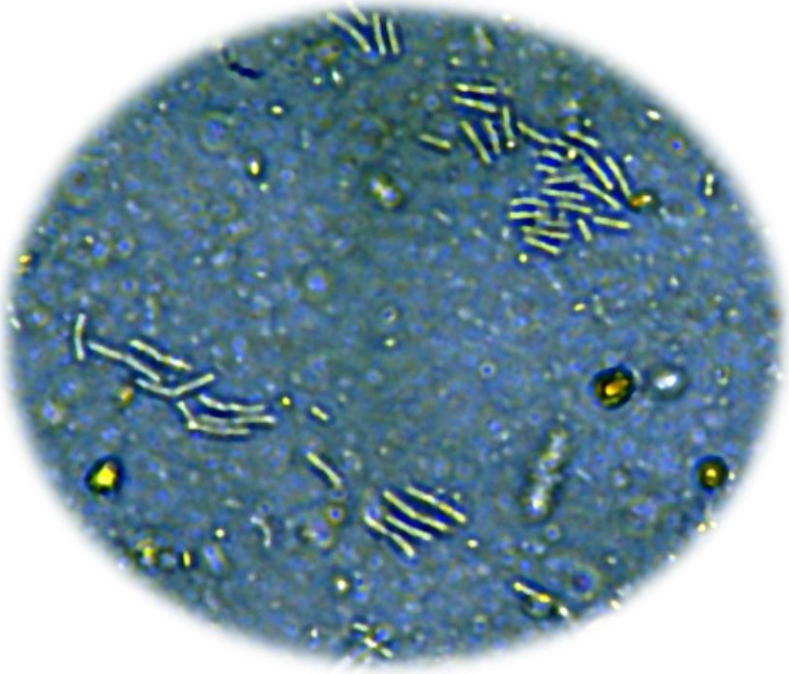

Fig 9.6 Large Rod-Shaped Bacteria found in group

Sr. No	Isolates	Morphology	Gram's Nature
1	Isolate 1	Large Rods in group	Gram positive
2	Isolate 2	Medium Rods in group	Gram positive
3	Isolate 3	Small Rods	Gram positive
4	Isolate 4	Small Rods in chain	Gram positive
5	Isolate 5	Medium Rods in chain	Gram positive
6	Isolate 6	Medium Rods in group	Gram positive
7	Isolate 7	Rods in group	Gram positive
8	Isolate 8	Cocci Separate	Gram positive
9	Isolate 9	Large Oval Shaped	Gram positive
10	Isolate 10	Medium rods in group	Gram positive
11	Isolate 11	Cocci in group	Gram positive
12	Isolate 12	Large Oval Shaped in Group	Gram positive
13	Isolate 13	Cocci in group	Gram negative
14	Isolate 14	Medium Rods	Gram negative
15	Isolate 15	Small Rods Separate	Gram negative
16	Isolate 16	Large Oval Shaped	Gram negative
17	Isolate 17	Medium Oval Shaped in Group	Gram negative

Table-1: Cultural and Morphological characteristics of the isolated microorganisms

Note: we perform Gram's staining method for Identify Gram positive or Gram negative

3.7.4 Microbial Consortium Development Process (Laboratory and Pilot Scale)

Fig. 10.1 Microbial Consortium Preparation at Initial Stage

Fig. 10.2 Developed microbial consortium after 36 days

Fig. 11 Developed Microbial Consortium for pilot scale study

Fig. 12.1 Pilot scale Microbial Consortium in initial Phase

Fig. 12.2 Pilot scale Microbial Consortium Preparation after 18 days

Fig. 12.3 Pilot scale Microbial Consortium Preparation after 36 days

Sr. No.	Trials of Developed Microbial Consortium	Days for Degradation of Floral waste
1	First trial	61
2	Second trial	54
3	Third trial	45
4	Fourth trial	38
5	Fifth trial	36

Table 2 Trials of Developed Microbial Consortium

3.8 Discussion:

By performing Gram's staining, the cultural and morphological characteristics of isolated microorganisms, total Seventeen microbial strains were isolated in which twelve is Gram Positive and five are Gram Negative strain as showed in table-1. Figure 9A to 9E also shows the shape and size of the microbial strain in which we found rod shaped, cocci shaped and Oval shaped bacteria and yeast. Table-2 saws the enrichment process of developed microbial consortium in which first trail consortium takes 61 days for degradation of floral waste and we continued with this similar microbial consortium for enrich process and after fifth trial it takes only 36 days which means consortia was enriched.