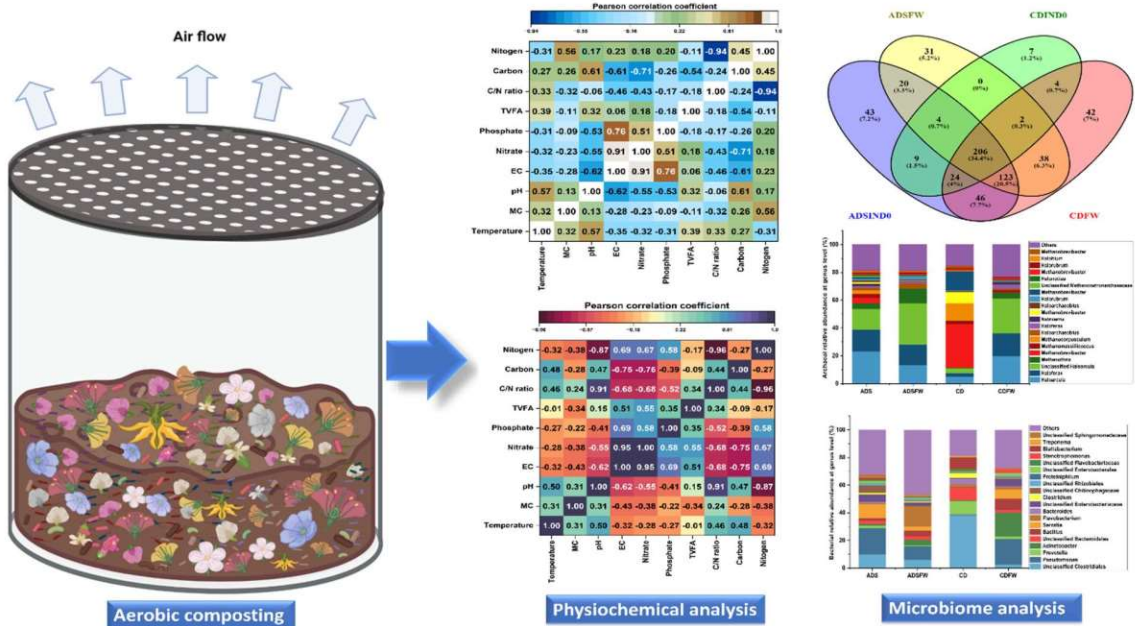


# Chapter 5

Evaluating the impact of digestate and raw manure on physicochemical conditions and microbiome dynamics during floral waste composting



Kushwaha, J., & Dhoble, A. S. (2024). Evaluating digestate and raw manure in floral waste composting: physicochemical properties and microbiome dynamics. *Environment, Development and Sustainability*, 1-19.

## 5.1 Background

Improper disposal of FW, particularly through open dumping, poses a significant environmental threat. It hampers the recreational value of water bodies and contributes to soil contamination upon discharge, mainly due to its rich nutrient composition. Such contamination disrupts the ecological balance and can have harmful impacts on the surrounding ecosystem [41]. The primary origins of FW include flower markets, places of worship, festivals, weddings, and similar events. For instance, India ranked second after China, generating approximately  $4.74 \times 10^6$  t/d of FW [96]. Around 2/5th of the daily flower production in Sri Lanka and India remains unsold, resulting in significant wastage [86]. Composting offers the optimal solution for managing FW. However, current studies mainly focus on kitchen waste, food waste, etc., leaving research on FW composting limited [96]. By converting FW into compost, the agricultural sector can derive benefits from the valuable macronutrients (such as nitrogen, phosphorus, potassium, and calcium) and micronutrients (including manganese, iron, copper, and zinc) that are present in the waste [97,98]. In addition to composting, several innovative and community-driven practices are being implemented across India for the sustainable management of floral waste. For example, the Tirupati Municipal Corporation recycles over six tonnes of floral waste daily, converting it into reusable products such as incense sticks, incense cones, bamboo-less incense, and havan cups through the Tirumala Tirupati Devasthanam agarbatti (incense) manufacturing plant. This initiative not only supports environmental sustainability but also provides employment to approximately 150 women from self-help groups. The products are packaged using recycled and plantable paper embedded with Tulsi seeds, contributing to a low-carbon and eco-conscious approach [98]. Similarly, in Ujjain, floral waste collected from the Mahakaleshwar Temple is processed at a three-tonnes-per-day facility into eco-friendly briquettes and compost. This initiative has engaged sixteen women from the Shiv Arpan Self-Help Group in the production of high-quality products from floral

waste. According to the Ujjain Smart City 2022 report, over 2,200 tonnes of floral waste have been treated, resulting in the production of more than 30 million incense sticks. These examples highlight the growing importance of decentralized, socially inclusive, and environmentally responsible approaches to floral waste management beyond composting. Microbes play a vital role in the composting process, facilitating it across various temperature phases: the thermophile phase (45–80 °C), hyperthermophile phase (>80 °C), mesophile phase (15–45 °C), and psychrophilic phase (>15 °C) [70]. Previous studies have identified dominant phyla, such as *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, known for their efficient degradation of complex organic matter, including cellulose, hemicellulose, lignin, chitin, and xylan. Additionally, species like *Acinetobacter*, *Bacillus*, *Stenotrophomonas*, *Luteimonas*, and *Pseudomonas* have been found to exhibit nitrogen-fixing and phosphate-solubilizing activities, promoting plant growth [70,71]. However, there is currently a lack of comprehensive studies on the core microbiomes of FW composting and the impact of different inoculum sources on it. The present study focuses on investigating aerobic composting of FW using CD versus ADS as inoculum, aiming to comprehensively understand core microbiomes and their associated physicochemical properties. The study aims to assess inoculum selection between ADS and CD, evaluating their respective microbial dynamics and physicochemical properties during FW composting. The hypothesis underlying this research is that combining process parameters with microbial dynamics can provide valuable information on the bioconversion of FW into effective compost.

### **5.1.1 Composting materials**

Fresh ADS was obtained from a local biogas plant situated at coordinates 25° 11' 33.95" N and 82° 51' 19.06" E in Varanasi, India. FW was collected from the prominent Vishwanath temple, located at coordinates 25° 16' 00.18" N and 82° 59' 18.56" E within the premises of Banaras Hindu University (BHU) in Varanasi. The FW used in the experiment consisted of 98%

marigold (*Tagetes erecta*), 1% rose (*Rosa rubiginosa*), 0.5% lotus (*Nelumbo nucifera*), and 0.5% pinwheel flower (*Tabernaemontana divaricate*). RS was harvested at the end of the growing season from an agricultural farm at Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India, in November 2022. After harvesting, the RS was mechanically cut into smaller pieces using an electric fodder-cutting machine. It was then promptly transported to the laboratory and stored in zip lock bags at room temperature. The RS underwent a two-step drying process for moisture reduction. Initially, it was subjected to natural air drying to eliminate the excess moisture. Subsequently, it was dried at 50°C in a hot air oven (Equitron, Stream Series) until a consistent weight was achieved. The dried RS was then further processed into smaller sizes (2 - 3 cm) using an electrical grinder (Philips Supreme 550W). The characteristics of inoculum and raw material are described in Table 5.1.

**Table 5.1.** Characteristics of raw material

<b>Characteristics*</b>	<b>Anaerobic Digester Slurry (ADS)</b>	<b>Cow Dung (CD)</b>	<b>Floral Waste (FW)</b>	<b>Rice straw (RS)</b>
Total Solids (TS, %wt)	7.47 ± 0.75	18.3 ± 0.65	88.11 ± 0.56	93.50 ± 0.19
Volatile Solids (VS, %wt)	4.45 ± 0.45	14.83 ± 0.53	82.35 ± 0.65	84.10 ± 0.37
VS/TS	0.59 ± 0.62	0.80 ± 0.87	0.94 ± 1.16	0.89 ± 0.02
Moisture content (%)	92.52 ± 0.75	81.70 ± 0.65	10.82 ± 1.12	6.49 ± 0.19

\* Numbers are mean ± standard deviation from three replicates

### 5.1.2 Experimental setup and design

Aerobic composting was performed in cylindrical vessels with a height of 35 cm, a diameter of 34 cm, and a total surface area of 5554.33 cm<sup>2</sup>. These vessels had a capacity of 20 liters and featured 104 evenly distributed 7 mm diameter holes across the entire surface, including the bottom and upper sections. Inside each vessel, a 35 cm long, 2.5 cm diameter air pipe with 4 mm diameter holes was installed at the center and connected to the air pump. The air pump

supplied a daily airflow of 0.6 L/min for ten minutes, promoting aerobic microbial activity. The vessels used in the experiment had a ratio of 12:5:2 FW:CD: RS, and the other with ADS replacing CD [71]. RS was employed as a bulking agent to arrest leaching and maintain the aerobic condition [71]. The compost moisture content was adjusted to 65% by adding deionized water. The mixture was turned every two days using a forklift during the 60-day cycle, and 10 g samples were collected every five days from distinct parts.

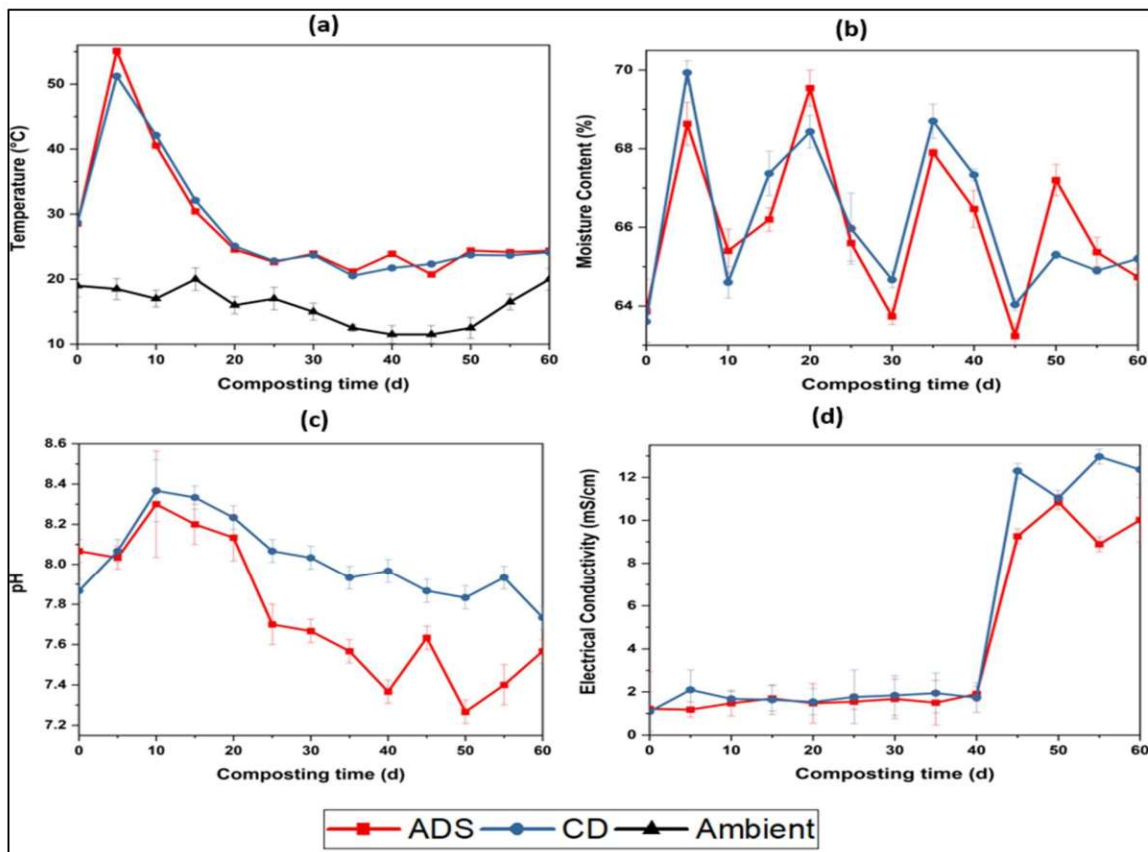
## **5.2 Results and discussion**

### **5.2.1 Physicochemical analysis**

Raw FW and RS contained significant amounts of cellulose, 39.26%, and 43.3%, respectively, which were essential for microbial degradation, while no cellulose data was available for ADS and CD. Hemicellulose content was 28.48% in FW and 36.37% in RS for ADS and CD. Lignin, a complex polymer that hindered microbial digestion, was present in lower amounts in FW (3.10%) compared to RS (8.528%). The high silica content in RS (45.60% of ash) could have created challenges in processing due to residue buildup, while silica data was unavailable for the other materials. In terms of energy potential, carbon content was highest in CD (42.06%), followed by RS (41.38%) and FW (39.80%), with ADS having the lowest carbon content at 29.27%. Hydrogen content, important for energy recovery, was highest in RS (6.43%) and lowest in FW (4.76%). Nitrogen, essential for microbial growth, was present at less than 1% in ADS and CD, suggesting that nitrogen supplementation might have been needed. However, FW contained 3.147% nitrogen, making it more suitable for nitrogen-dependent microbial processes.

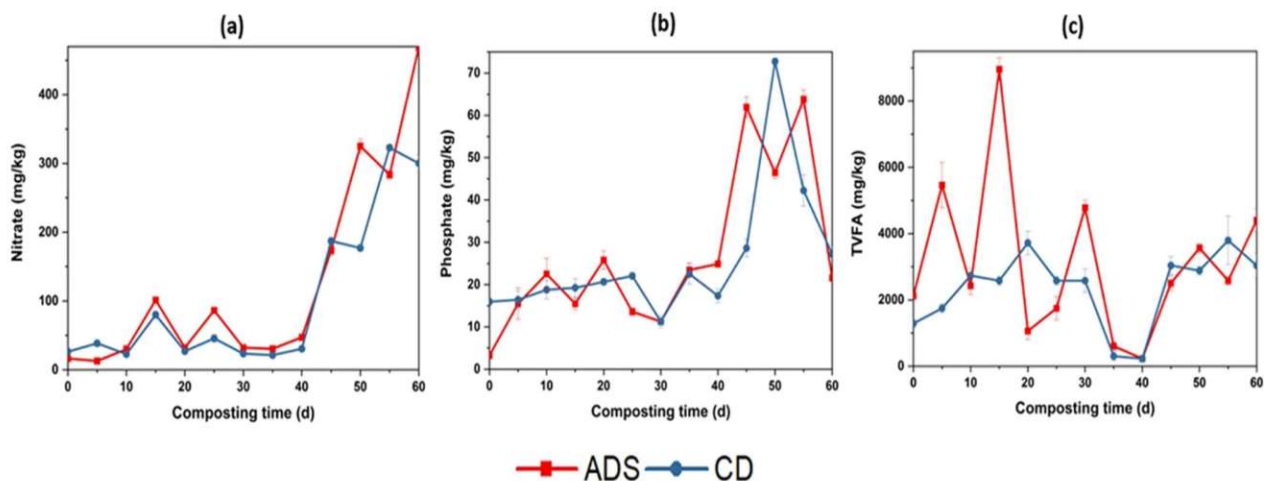
Temperature is crucial for composting, as it strongly influences microbial activity and decomposition [75]. *Figure 5.1. (a)* shows temperature measurements for ADS, CD, and ambient room temperature at different time points. The initial ambient temperature of 19°C on Day 0 rose significantly to 55.12 °C (ADS) and 51.22 °C (CD) on Day 5, indicating the

transition to the thermophilic phase. Despite a decrease in ambient temperature to 17 °C on Day 10, both treatments maintained relatively high temperatures (40.55 °C for ADS and 42.12 °C for CD), indicating favorable conditions for microbial degradation action. Moisture content affects composting and temperature dynamics. Fluctuations in moisture percentages are seen in *Figure 5.1. (b)* showed corresponding variations. Higher moisture percentages on days 5 and 10 correlated with elevated temperatures [77]. pH values in *Figure 5.1 (c)* exhibited fluctuations and increased during composting, peaking at 8.37 (CD) and 8.30 (ADS) on the tenth day. The pH initially increased and then decreased, which might have been influenced by microbial activity and organic matter decomposition [75]. Adequate moisture supported microbial activity and facilitated organic matter breakdown, contributing to heat generation during composting.



**Figure 5.1.** (a) Temperature, (b) Moisture Content, (c) pH, (d) Electrical conductivity in aerobic composting of floral waste with cow dung (CD) and anaerobic digester slurry (ADS)

The electrical conductivity measurements of CD and ADS samples during composting are observed in *Figure 5.1. (d)*, provided insights into changes in ion concentration and nutrient availability, such as potassium, ammonium, and phosphate [52]. Both samples initially had low electrical conductivity values (CD: 1.09 mS/cm, ADS: 1.22 mS/cm), which gradually increased over time. After ten days, CD reached its peak at 1.68 mS/cm, whereas ADS peaked at 1.48 mS/cm. A significant increase occurred after day 40, with values of 12.30 mS/cm (CD) and 9.25 mS/cm (ADS), which might indicate ion and nutrient accumulation. The initial nitrate levels were 26.44 mg/kg for CD and 16.43 mg/kg for ADS. Before decreasing, they peaked at 322.97 mg/kg for CD and 468.74 mg/kg for ADS. After day 40, nitrate and phosphate concentrations fluctuated, indicating nutrient availability and organic matter decomposition, as observed in *Figure 5.1. (a, b)*.



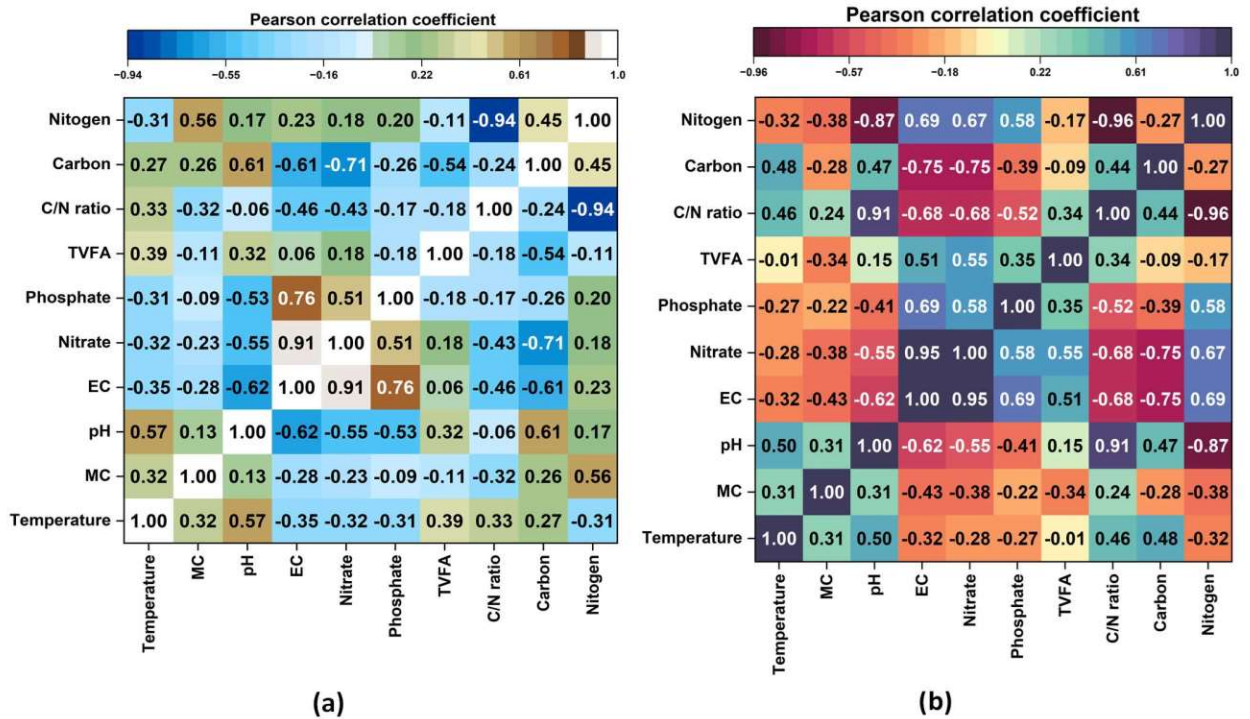
**Figure 5.2.** (a) Nitrate (b) Phosphate (c) Total Volatile Fatty Acids (TVFAs), in aerobic composting of floral waste with cow dung (CD) and anaerobic digester slurry (ADS)

Correlations between parameters putatively revealed potential associations, such as positive correlations between nitrate and phosphate, nitrate and TVFA, and phosphate and TVFA in *Figure 5.2 (a, b, c)*. In the presented study, carbon content decreased throughout the composting period. In contrast, nitrogen content seemed to vary, with higher levels observed at specific

time points. The carbon content decreased while the nitrogen content showed variable patterns. Both CD and ADS samples initially had higher C/N ratios, indicating higher carbon content. As composting progressed, carbon content decreased, suggesting the breakdown of carbon-rich organic matter [79]. Nitrogen content remained consistently low in CD (1% to 3.08%) and exhibited more variability in ADS (1% to 4.35%). Around day 20 and 50 in ADS, a relatively higher nitrogen content was observed, while the C/N ratio was lower. This suggested there may be the release of nitrogen during decomposition despite a decrease in carbon content [78]. pH measurements indicated microbial activity and organic matter decomposition, while electrical conductivity reflected changes in nutrient availability [99]. Amino acid levels rose due to microbial enzymatic activity but could decrease due to humic substance production or nitrogen utilization [75]. These findings demonstrated the dynamic nature of composting, highlighting the interactions between organic matter decomposition, nutrient transformation, and microbial activity [77]. After 40 days, there might be an increase in organic matter decomposition, nutrient mineralization, and microbial activity, which could result in elevated ion and nutrient accumulation. This increase in microbial activity may lead to higher EC, nitrate, and phosphate [100]. These elevated levels could indicate the release of nutrients resulting from the breakdown of organic matter. Additionally, positive correlations might exist between nitrate and phosphate levels and between nitrate and TVFA, further supporting the increased microbial activity [94].

The Pearson correlation analysis for ADS treatment during composting revealed several significant associations in *Figure 5.3. (a)*. Temperature positively correlated with pH, moisture content (MC), and C/N ratio with a p-value less than 0.05 [71]. Additionally, EC is negatively correlated with pH and positively correlated with nitrate. The C/N ratio had weak positive correlations with temperature and MC but a strong negative correlation with carbon [76]. Moreover, carbon was moderately correlated with pH and strongly negatively correlated with

the C/N ratio. Similarly, in CD treatment during composting, as shown in *Figure 5.3*. (b) temperature was found to be positively correlated with pH and negatively correlated with EC [77]. pH exhibited positive correlations with EC, nitrate, and phosphate, while EC demonstrated positive correlations with nitrate and phosphate [78]. These correlation patterns provide valuable insights into the interdependencies and influences among the variables in the ADS and CD treatment composting process.

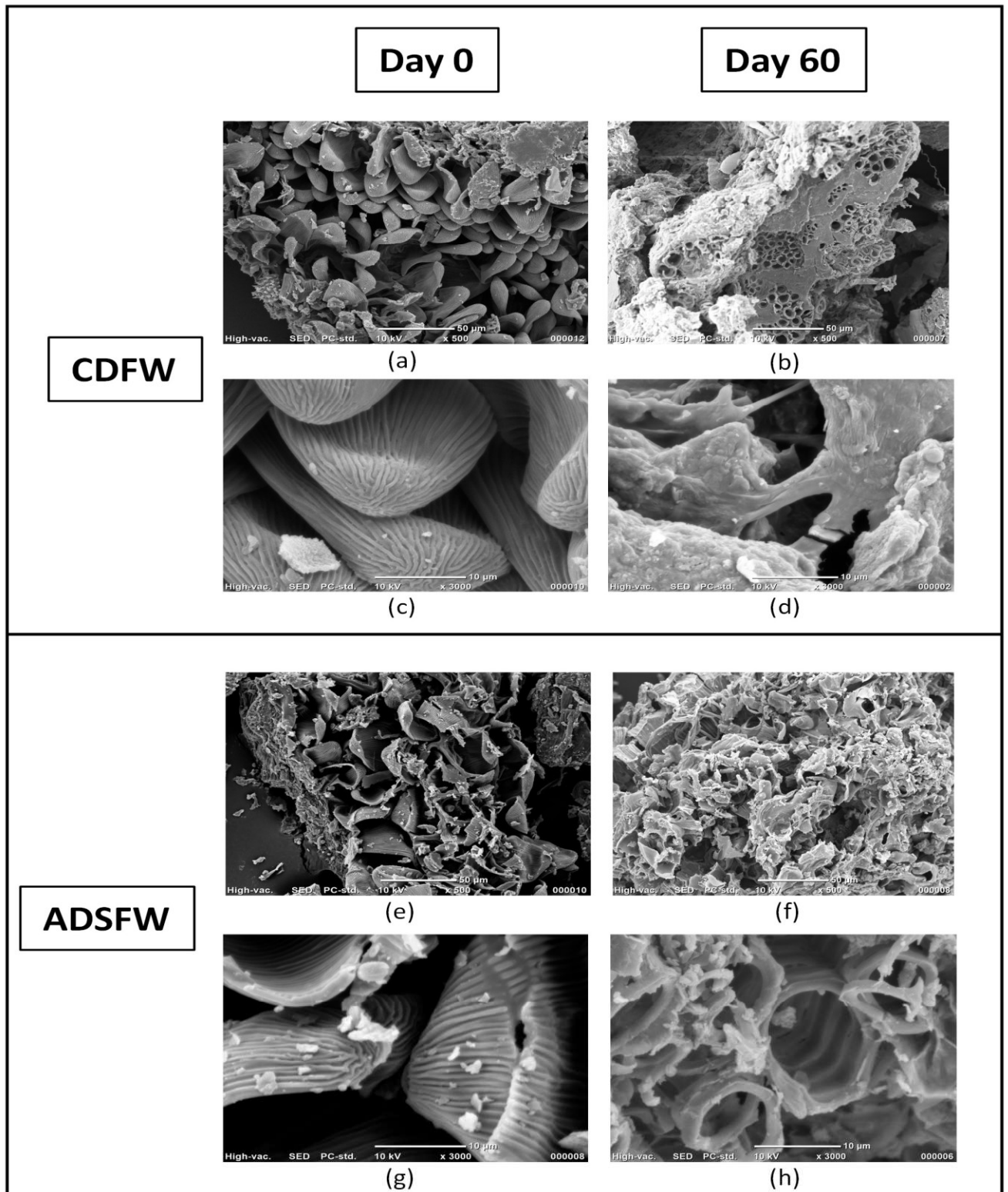


**Figure 5.3.** Pearson correlation analysis of physiochemical properties (a) anaerobic digester slurry (ADS), (b) cow dung (CD).

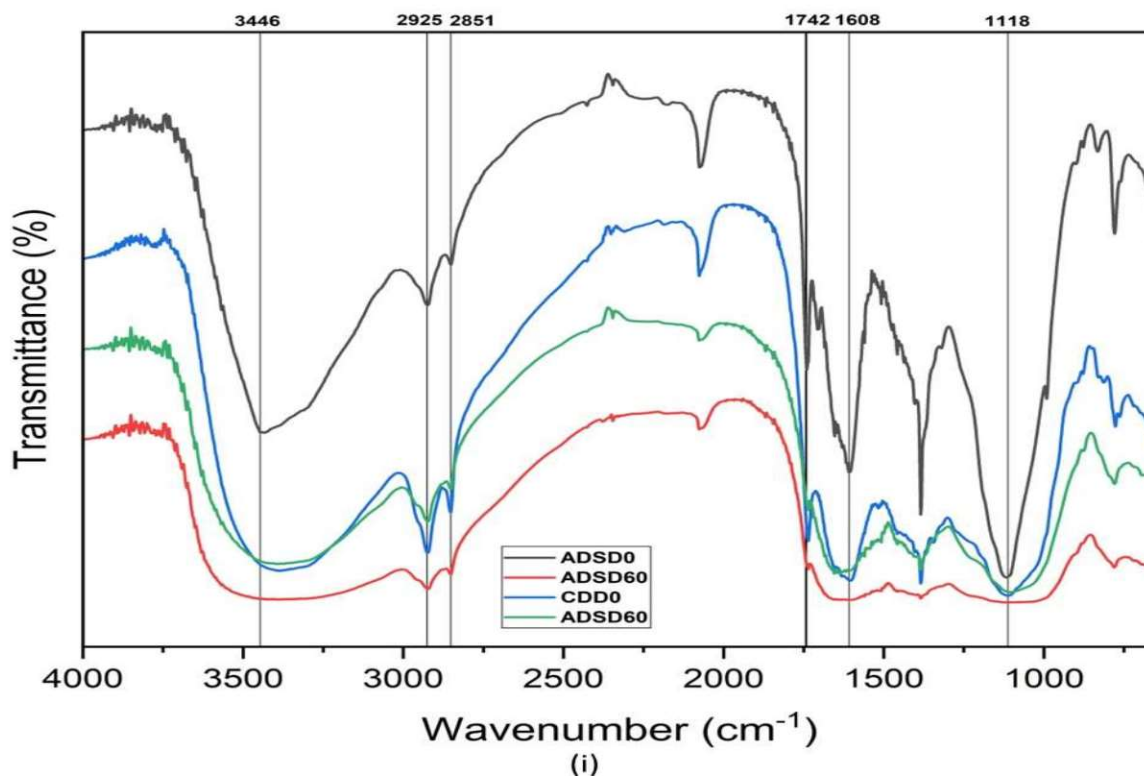
### 5.2.2 FTIR and BT-SEM analysis

FTIR analysis assessed the impact of inoculum on the chemical composition and structure of composted FW (4000-400  $\text{cm}^{-1}$  range). Comparisons were made between the FTIR spectrum of ADSD0, CDD0, ADSD60, and CDD60, as shown in *Figure 5.5*. Higher frequency measurements showed a band at 3446  $\text{cm}^{-1}$ , indicating stretching vibrations of OH hydroxyl groups. The wave number is smaller, and the profile is more expansive, suggesting the presence

of hydrogen bonding interactions compared to free OH hydroxyl. This band has been linked to carboxylic acids and hydroxyl phenols in the context of composting FW[82], as well as N-H stretching and hydroxyl phenols [85,102]. The spectra match these results since the band's asymmetries indicate the presence of the OH and NH groups at  $3446\text{ cm}^{-1}$  and  $3446\text{ cm}^{-1}$ , respectively. Due to amidic groups from FW, FTIR analysis of compost samples shows a peak in the wavenumber range of  $1650\text{-}1600\text{ cm}^{-1}$ , particularly at  $1608\text{ cm}^{-1}$  [103]. These peaks could be related to molecules resulting from microbial activity and display deviations in ADSD0, ADSD60, CDD0, and CDD60 [96]. For all four samples, there was a significant difference in the peak heights at  $1118\text{ cm}^{-1}$ , which suggests that the C-N and C-H bonds of aliphatic amines and alkyl halides have changed [84]. The two measured peaks, at  $2925\text{ cm}^{-1}$  and  $2851\text{ cm}^{-1}$ , represent the C-H methyl and aliphatic methylene groups. The microbial oxidation of aliphatic and peptide carbon chains is reflected in these bands, which are specific to C-H aliphatic molecules and whose intensity decreases with co-composting [85].



**Figure 5.4.** Images of SEM micrographs of floral waste (FW) compost at day 0 (a, c) and day 60 (b, d) with cow dung (CD) at 50X and 10X, and floral waste (FW) at day 0 (e, g) and day 60 (f, h) with anaerobic digestate slurry (ADS) at 50X and 10X



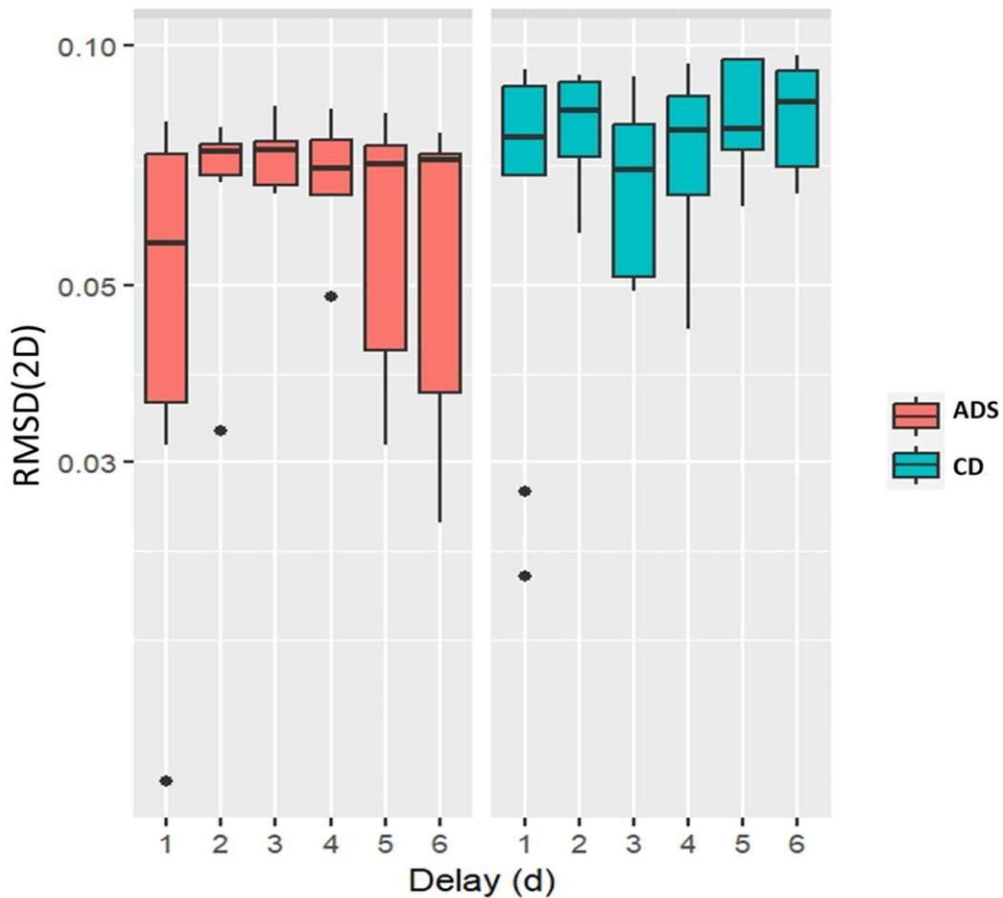
**Figure 5.5.** FTIR Spectra of ADSD0 and CDD0 (Day 0 samples) compared to ADSD60 and CDD60 (Day 60 samples).

Images of Scanning Electron Microscopy (SEM) of FW compost are shown in *Figure 5.4.* at day 0 (a, c) and day 60 (b, d) with CD at 50X and 10X, and FW at day 0 (e, g) and day 60 (f, h) with ADS at 50X and 10X. In the SEM images of FW samples, intricate ridges and grooves can be observed. The FW creates a wavy surface filled with organic matter, and its unevenness suggests that FW might contain less lignin [89]. Notably, after 60 days, visible pore spaces were only observed in the composting sample ADS, distinguishing it from the manure-based composting sample CD when viewed at 50X and 10X magnifications. This suggests that ADS-based composting might have more decomposition potential, making it a valuable option for FW composting [106].

### 5.2.3 Flow cytometry analysis of microbial population

Autocorrelation analysis of consecutive days' data (time points 1 to 6) provided valuable insights into the continuous and dynamic nature of microbial dynamics and the impact of

perturbations as shown in *Figure 5.6*. A delay referred to the time lag between a day's data and its lagged values from previous days. For example, with a 10-day delay, this compared each day's data (e.g., D1) with the data from the previous day (e.g., D0) to uncover patterns or dependencies between consecutive days. Distance matrix computations were used to identify microbial succession patterns, while autocorrelation analysis revealed shifts and temporal dependencies within populations [85]. Additionally, the extremum in distance evaluated phenotype stability, while disturbance magnitude assesses the behaviour in the anaerobic community [57]. ADS analysis showed a significant perturbation spike at delay 2, indicating substantial alterations in the microbial community. Delays 4, 5, and 6 exhibited similar dynamics with ongoing perturbation and stable dissimilarity values, suggesting no significant changes based on RMSD differences.



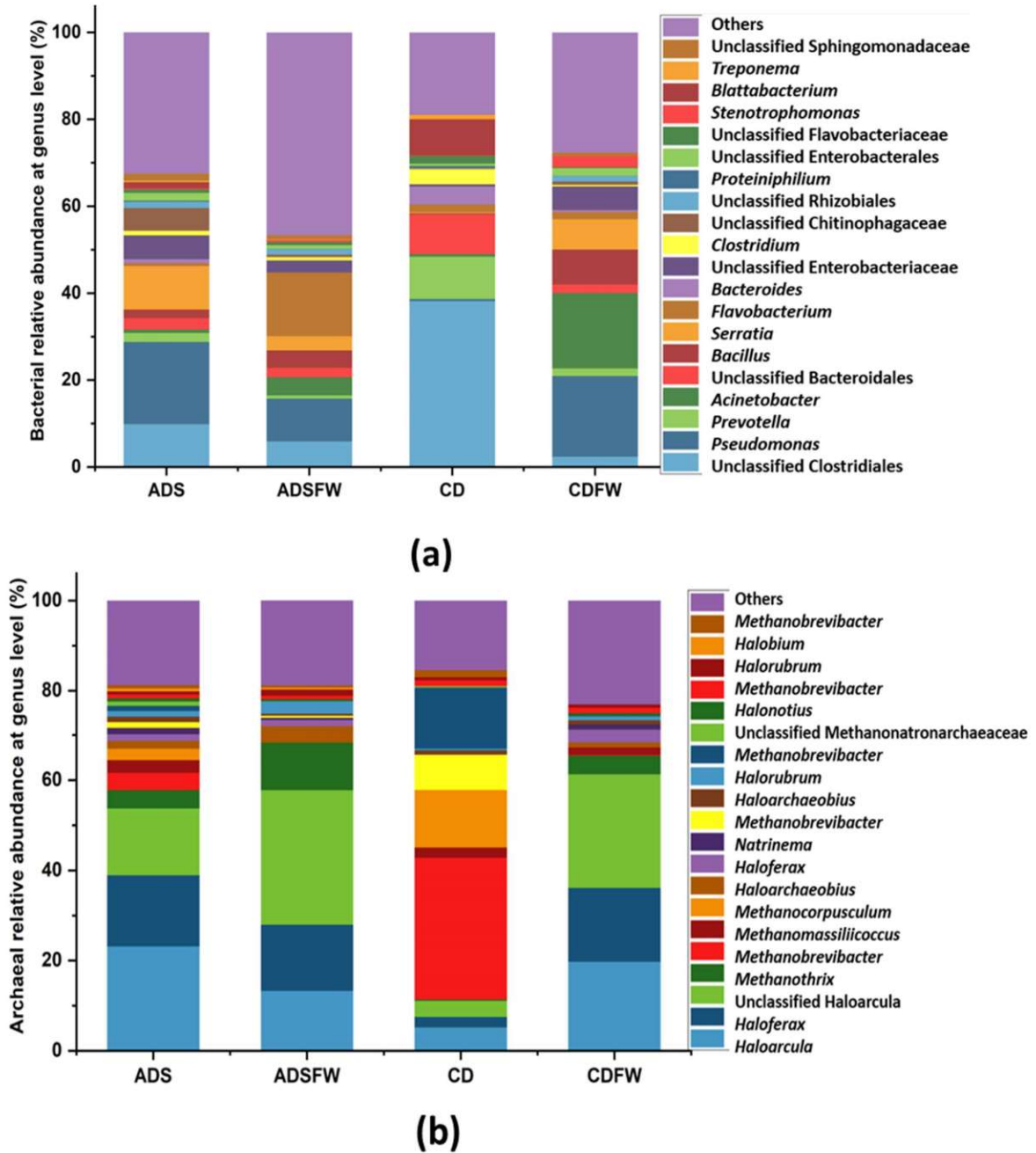
**Figure 5.6.** Autocorrelation plot for dissimilarity RMSD (2D) vs Delay

Persistent changes in forward scatter area (FSC-A) and side scatter area (SSC-A) distances reflected evolving microbial dynamics. CD compost analysis displayed dynamic changes, with a minor difference at delay 2 and a noticeable decrease at delay 3. Delays 4 and 5 in CD showed similar dynamics as in ADS. These microbial community patterns inform composting dynamics and their impact on physiochemical properties, enhancing understanding of microbial responses to experimental conditions.

#### **5.2.4 Microbial community diversity assessment**

During composting, the microbial composition plays a crucial role in the decomposition and nutrient cycling processes. The genera *Pseudomonas*, *Bacillus*, *Streptomyces*, *Prevotella*, *Serratia*, *Luteimonas*, and *Flavobacterium* were observed in all samples in *Figure 5.7 (a)*. *Bacillus* exhibited a significant increase of 63.35% in CDFW mature compost compared to its initial inoculum, CD, while ADSFW mature compost showed a minor increase of 0.17%. *Streptomyces* increased by 129.5% in CDFW and by 8.68% in ADSFW. *Luteimonas* increased by 12.5% in CDFW and by 1.68% in ADSFW. *Corynebacterium* showed a 5.4% increase in CDFW, whereas ADSFW experienced a decrease of 0.12%. These genera were also dominant in the denitrification process, indicating simultaneous nitrification and denitrification. This suggests that certain microbial groups were involved in converting ammonia to nitrate (nitrification). In contrast, others, including *Bacillus*, *Streptomyces*, *Luteimonas*, and *Corynebacterium*, were responsible for the conversion of nitrate back to nitrogen gas (denitrification) [90]. The coexistence of these genera during nitrification implies a potential coupling of these essential processes in the nitrogen cycle [97]. *Bacillus* underwent heterotrophic nitrification and aerobic denitrification [89,90]. A higher abundance of *Bacillus* was found in ADSFW, CDFW, ADS, and the last CD, indicating that these composts likely supported the growth and activity of *Bacillus* species. Conversely, *Pseudomonas* participated in the denitrification and accumulation of polyphosphate and was related to the degradation

and nitrification of aromatic hydrocarbons [108]. Furthermore, *Pseudomonas* and *Bacillus* worked together for nitrogen fixation [109]. The higher abundance of *Pseudomonas* observed in the ADS sample suggests that they provide favorable conditions for organic matter decomposition and nutrient cycling, potentially leading to increased *Pseudomonas* activity.



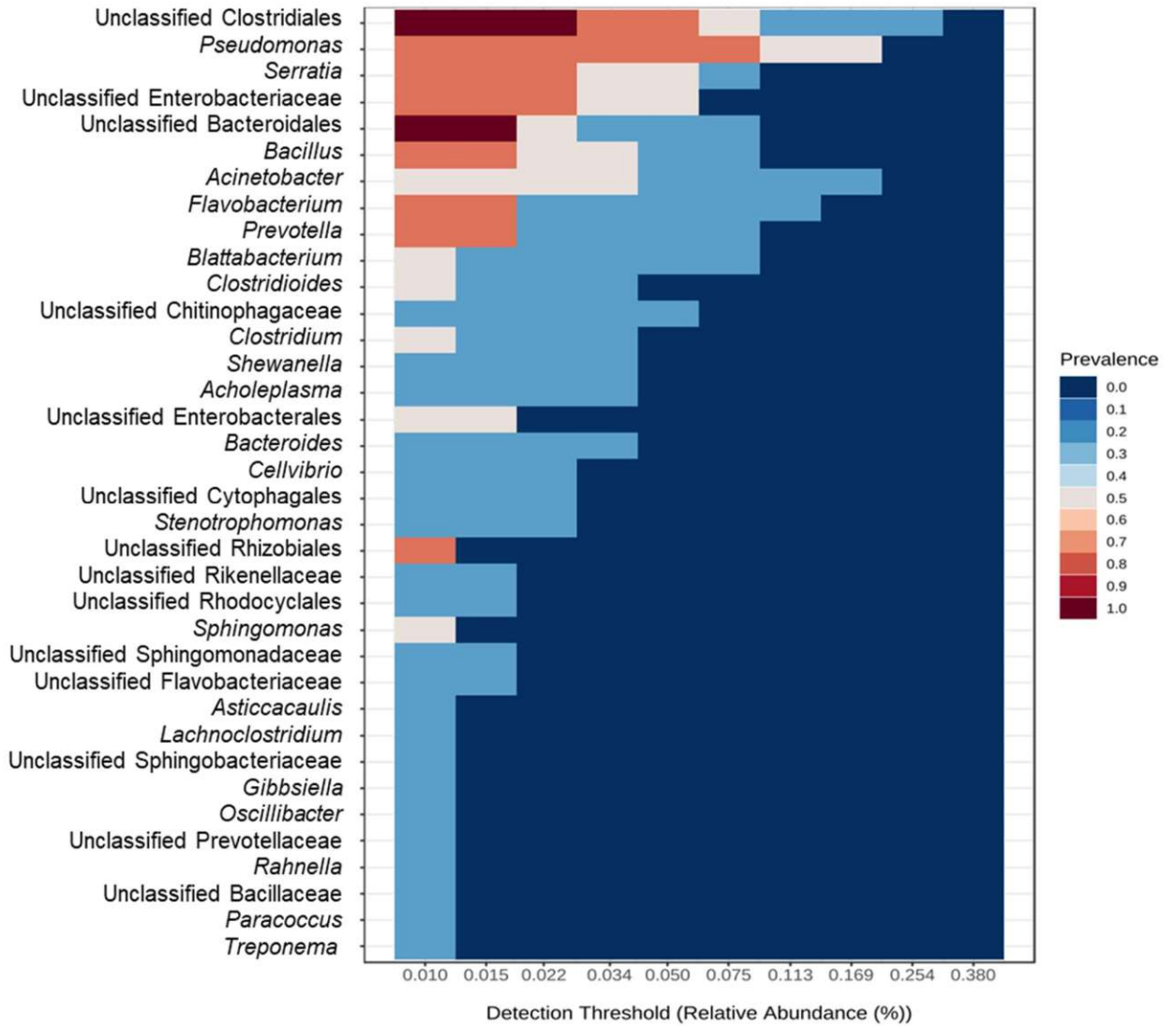
**Figure 5.7.** Analysis investigated the composition of bacterial (a) and archaeal communities (b), specifically the top 20 genera, before and after composting floral waste with cow dung (CDFW) and anaerobic digester slurry (ADSW) as inoculum, compared to raw inoculum samples CD and ADS.

*Serratia* produces alkaline proteases that exhibit stability over a broad temperature range, similar to the enzymes produced by *Bacillus* spp. [99]. These genera were present in significant amounts in both ADSFW and CDFW. *Flavobacterium* contributed to composting by degrading organic substrates, releasing nutrients, and facilitating the breakdown of organic matter, which was most prominent in ADSFW [100]. *Clostridium* bacteria were well-known for their involvement in anaerobic decomposition processes. The similar abundance of *Clostridium* found in CDFW and ADSFW samples indicates the presence of anaerobic degradation in some parts of the compost. This suggested that *Clostridium* could thrive and contribute to the decomposition of organic matter in these environments [90, 87]. Furthermore, *Prevotella* is commonly used as an indicator of pathogen reduction in both nitrification and denitrification processes [88]. The higher abundance of *Prevotella* in the CD and CDFW, whereas ADS and ADSFW showed comparatively less, suggested that this specific compost may have contained a more significant proportion of plant material or recalcitrant organic matter [88]. This provided an environment conducive to *Prevotella* growth and its involvement in the breakdown of complex carbohydrates. In summary, the presence and abundance of *Pseudomonas*, *Bacillus*, *Clostridium*, *Lactobacillus*, and *Prevotella* in composting systems indicated their potential contributions to organic matter decomposition, nutrient cycling, and the breakdown of complex compounds. Throughout the composting process, a total of 31 phyla were detected. Among all phyla, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Spirochaetes*, *Chloroflexi*, and *Planctomycetes* were identified as the dominant ones. The dominance of *Proteobacteria* and *Firmicutes*, known for their versatile metabolism and ability to degrade various organic substances [60]. *Bacteroidetes* and *Actinobacteria* also contributed significantly to the breakdown of complex carbohydrates and lignocellulosic materials [89]. The presence of dominant microbial genera like *Haloarcula*, *Haloferax*, and *Unclassified Haloarcula* indicates their active involvement in the decomposition process in Figure 5.7. (b). *Haloarcula* and

*Haloferax* excelled in high-salt environments and were proficient in breaking down complex organic compounds [90]. *Haloarchaeobius* and *Halorubrum*, found in both inoculum CD and ADS samples, likely contributed to organic matter breakdown, especially in the high-salt environment of CD composting [90]. CDFW had a high abundance of *Haloarcula* and *Haloferax*, accelerating organic matter decomposition. In ADSFW, *Haloarcula* and *Haloferax* played active roles in organic matter breakdown, while methane-producing archaea decreased significantly in both CDFW and ADSFW, indicating aerobic conditions were maintained.

### 5.2.5 Microbiome comparative analysis

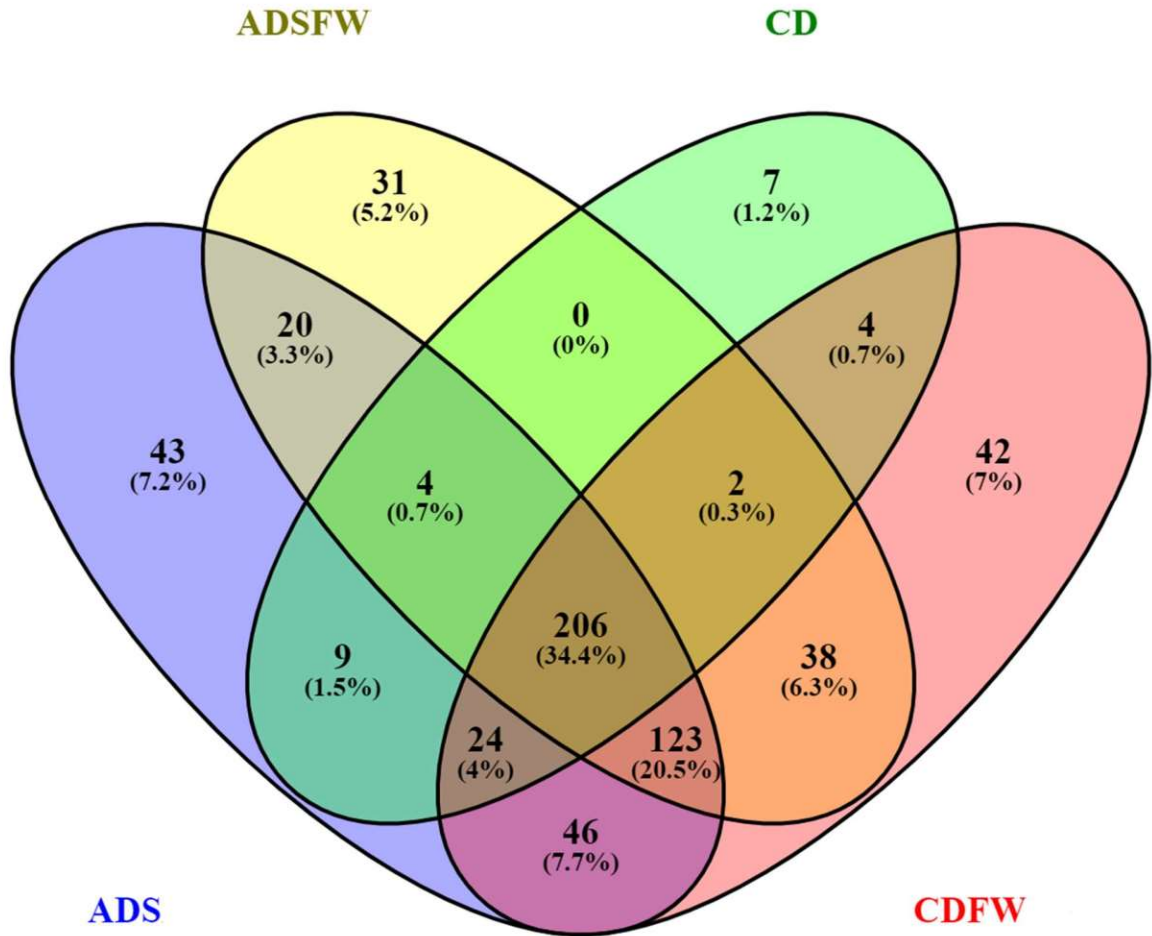
The prevalence of microbial taxa and core microbiomes is substantial in composting. Composting involves the decomposition of organic materials by microorganisms, forming nutrient-rich humus. These microorganisms constitute the core microbiomes of the composting system. Prevalence data provides insights into the significance of specific microbial genera in composting, as shown in *Figure 5.8*. Genera with a prevalence of 1, such as *Unclassified Bacteroidales* and *Unclassified Clostridiales*, were highly abundant and widely distributed, indicating their critical ecological roles. Genera with a prevalence of 0.75, including *Unclassified Rhizobiales*, *Unclassified Enterobacteriaceae*, *Flavobacterium*, *Serratia*, *Bacillus*, *Prevotella*, and *Pseudomonas*, were relatively abundant and contributed significantly to microbial dynamics. Moderate prevalence genera like *Sphingomonas* and *Clostridioides* also had notable impacts. Genera with lower prevalence, such as *Shewanella* and *Treponema*, played specialized roles. The absence of *Aminobacterium* suggested negligible influence. Considering the prevalence of these genera enhanced our understanding of their contributions to organic matter decomposition, nutrient cycling, and composting dynamics.



**Figure 5.8.** Core microbiomes heatmap illustrates microbial communities' shared genera in the core composition.

Rarefaction curves of these microbes revealed that CDFW had the highest richness, followed by ADS, ADSFW, and CD. As the initial inoculum, CD displayed the lowest richness and taxa count, indicating a less diverse microbial community than ADS but shared specimens with all. The higher richness in mature compost (CDFW) suggested the evolution of diverse composting microbes, promoting breakdown, stability, and nutrient composition. As the initial inoculum for composting, ADS showed relatively high richness, indicating diverse and effective

composting microbes. Several noteworthy observations were made when comparing the unique and shared Operational Taxonomic Units (OTUs) among the samples in the context of composting microbes.



**Figure 5.9.** Venn diagram showing the distribution of shared bacterial OTUs among four samples: ADS and CD at day 0, and ADFW and CDFW at day 60

Each sample exhibited a discernible set of exclusive OTUs, representing specific microbial taxa unique to that particular sample. For instance, inoculum ADS was characterized by 43 unique OTUs, ADFW had 31 unique OTUs, inoculum CD had 7 unique OTUs, and CDFW had 42 unique OTUs, as shown in *Figure 5.9*. These exclusive OTUs played a pivotal role in shaping the distinct microbial composition of each sample. Conversely, intriguing patterns

emerged upon analyzing the shared OTUs among different sample combinations. For instance, ADS, ADSFW, and CD exhibited 43 shared OTUs, indicating a substantial overlap in microbial composition among these samples. Similarly, ADS and CD shared 9 OTUs, CD and CDFW shared 4 OTUs, and ADSFW, CD, and CDFW shared 4 OTUs. These shared OTUs signified similarities and potential interactions within the microbial communities of the respective samples. Furthermore, certain combinations of samples demonstrated a substantial number of shared OTUs. For example, ADS, ADSFW, CD, and CDFW collectively shared 206 OTUs. Comparative analysis of unique and shared OTUs revealed distinct microbial profiles and cooperative interactions in composting, promoting organic substrate decomposition [73].

### **5.2.6 Alpha and beta diversity analysis of bacterial community**

Alpha diversity measures the richness and relative abundance of bacteria in a sample [91]. Table 5.2. showed different indices representing alpha diversity. The Chao 1 index indicated richness, while the Shannon, Simpson, and Fisher indices reflected both richness and relative abundance. The Chao 1 index measured richness, and ADS had the highest value (390.5), followed by ADSFW (343.53), CDFW (373.03), and CD (240.2), indicating varying levels of species richness. The Fisher index reflected species diversity, and ADS had the highest value (56.13), followed by ADSFW (47.28), CDFW (52.69), and CD (28.85), indicating different levels of diversity. The Shannon index combined richness and evenness, and ADS (3.81) and ADSFW (3.91) exhibited significant diversity, while CD (2.62) had lower diversity. ADS had the highest number of reads (88,249) and exhibited higher richness and diversity, suggesting a greater variety of microbial species. ADSFW had slightly fewer reads (51,231) and slightly reduced richness and diversity compared to ADS. CD had the lowest number of reads (49,733) and lower richness and diversity. CDFW had the highest number of reads (96,704) and higher richness and diversity than CD. ADS and ADSFW showed higher species diversity and richness than CD and CDFW.

**Table 5.2.** Comparison of alpha diversity measures in samples

<b>Sample</b>	<b>Chao 1</b>	<b>Fisher</b>	<b>Shannon</b>	<b>Simpson</b>	<b>Number of reads</b>
<b>ADS</b>	390.50	56.13	3.81	0.93	88249
<b>ADSFW</b>	343.53	47.28	3.91	0.95	51231
<b>CD</b>	240.20	28.85	2.62	0.82	49733
<b>CDFW</b>	373.03	52.69	3.50	0.91	96704

Beta diversity analysis of composting microbes utilized Bray-Curtis dissimilarity to assess microbial community variation [60]. PC1 represented 43.2% shared species composition along the x-axis, while PC2 represented 35% shared species composition along the y-axis. Higher percentages indicated greater similarity in microbial composition within each respective axis. Sample coordinates displayed variations, suggesting differences in species composition. For example, ADSIND0 and CDFW showed negative values along PC1, indicating potential dissimilarity compared to ADSFW and CDIND0 with positive values. CDFW had a notably negative value along PC2, signifying potential distinction from other samples, while CDIND0 displayed the highest positive value along PC2, suggesting potential dissimilarity from other samples.

### **5.3 Challenges and future prospects**

Exploring the potential of FW and digestate in composting offers multifaceted advantages beyond waste reduction. Typically discarded, FW strains landfill and adds to greenhouse gas emissions. However, through composting, this waste becomes a valuable resource, diverting it from landfills. Simultaneously, digestate, a byproduct of anaerobic digestion, contains rich organic matter and beneficial diverse microorganisms, enhancing composting efficiency. This study investigates the symbiotic potential between digestate and FW in composting. While it compares digestate to raw manure, it might overlook opportunities to maximize their combined

effectiveness. The challenges associated with achieving safe and high-quality compost include addressing issues with pathogens, temperature regulation, odor control, and emissions. This necessitates meticulous adjustments in feedstock composition, moisture levels, nutrient balance, sanitation practices, and odor management. Future investigations could deeply analyze the microbiome dynamics within digestate paired with FW, employing techniques like metagenomics and flow cytometry. This exploration, especially at larger scales, is vital for enhancing composting efficiency and advancing sustainable waste management. Understanding intricate microbial interactions and their impact on compost quality drives the development of more effective large-scale composting strategies. FW composting shows promise, especially in pilgrimage cities where substantial FW accumulates. Studying the combined impact of these waste streams on microbial communities and compost quality could lead to innovative composting methods. This comprehensive approach aims to reduce environmental impact, maximize resource recovery, create high-quality compost, and improve sustainable waste management practices.

#### **5.4 Conclusion**

Both ADS and CD proved to be an effective inoculum source for FW composting. ADS showed a better microbial and archaeal community richness compared to CD. Increased carbon percentage, along with the increase in nitrate and phosphate concentration, confirms the core microbiome's role in compost maturation. *Bacillus* increased by 63.35% in CDFW compost compared to CD and by 0.17% in ADSFW. *Streptomyces* rose by 129.5% in CDFW and 8.68% in ADSFW, *Luteimonas* by 12.5% in CDFW and 1.68% in ADSFW, while *Corynebacterium* increased by 5.4% in CDFW and decreased by 0.12% in ADSFW. These genera are prominent in denitrification processes, indicating concurrent nitrification and denitrification during FW degradation. These findings demonstrate the fruitfulness of aerobic composting for FW management.