BIOGAS PRODUCTION FROM CO-DIGESTION OF PARSLEY (*petroselinumcrispum)* STEM WASTE AND FLORAL WASTE IN ANAEROBIC DIGESTION

APROJECT REPORT

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In

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BONAFIDE CERTIFICATE

Certified that this project report **"BIOGAS PRODUTION FROM CO DIGESTION OF PARSLEY (*PETROSELINUMCRISPUM)* STEM WASTE AND FLORAL WASTE IN ANAEROBIC DIGESTION"** is the Bonafide work of **"KEERTHANA P (620821214024) , SUPRIYA S (620821214056), KAVYA J (620821214024), LAVANYA S (620821214028)"** who carried out the project work under my supervision.

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INTERNAL EXAMINER **EXTERNAL EXAMINER**

DECLARATION

We affirm that the project work titled **"BIOGAS PRODUCTION FROM CO-DIGESTION OF PARSLEY (*PETROSELINUMCRISPUM)* STEM WASTE AND FLORAL WASTE IN ANAEROBIC DIGESTION "** being submitted in partial fulfillment for the award of **B.TECH. (Biotechnology)** is the original work carried out by us. It has not formed part of any other project work submitted for award of any degree or diploma, either in this or any other university.

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Gnanamani College of Technology, Namakkal-637018

Accredited by NBA&NAAC"A" Grade

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DEPARTMENTOFBIOTECHNOLOGY

Institute vision

Emerging as a technical institution of high standard and excellence to produce quality Engineers, Researchers, Administrators and Entrepreneurs with ethical and moral values to contribute the sustainable development of the society.

Institute Mission

We felicitate our students

- To have in-depth domain knowledge with analytical and practical skills in cutting edge technologies by imparting quality technical education.
- To be industry ready and multi-skilled personalities to transfer technology to industries and rural areas by creating interests among students in Research and Development and Entrepreneurship.
-

Department vision

To develop proficient biotechnologists through high quality education and promote scientific knowledge by research training to address the global challenges and lead advances in health care sector and industrial biotechnology.

Department Mission

- To educate Students to acquire strong knowledge and skills in Biotechnology.
- Tocreateopportunitiesformulti-disciplinaryeducationandresearchtowards industrial innovation.
- To emphasize and equip the students with critical thinking, analytical and communication skills.
- To cultivate effective and socially responsible Biotechnologists who can meet existing and emerging global challenges.

Program Educational Objectives(PEOs)

PEO-1	To prepare students to excel in research and to succeed in Biotechnology sector by training them with good scientific and technical knowledge.
PEO-2	The course has a multi disciplinary approach, and therefore the student is able to choose various options in Nano technology, Pharmaceutical technology and Food Technology.
PEO-3	To develop excellence in leadership skills, respect for authority, loyalty and the life-long learning needed for a successful scientific and professional career.

Program Outcomes (POs)

PO-1	**Engineering knowledge:** Graduates will demonstrate good knowledge of Statistics, Science and Technology to solve engineering and research problems
PO-2	**Problem analysis :** They will be able to demonstrate and able to independently perform experiments in areas of Bioprocess, Enzyme Technology ,Genetic Engineering ,Animal Biotechnology and Immuno technology
PO-3	**Design/development of solutions :**They will be able to design and conduct experiments, analyze and interpret data.
PO-4	**Conduct investigations of complex problems:** The graduates will be capable of demonstrating an ability to design an experiment, component or process as per needs and specifications.
PO-5	**Modern tool usage:** The graduate will be adept at performing experiments in cutting edge areas of emerging biotechnology.
PO-6	**The engineer and society :** Conduct themselves to up hold the professional and social obligations
PO-7	**Environment and sustainability:** Design the system with environmental consciousness and sustainable development.
PO-8	**Ethics:** Interact with industry, business and society in a professional and ethical manner They willed monstrate the ability and requirements to sense the needs of the nation and their role in Nation building.
PO-9	**Individual and team work :** Function effectively as an individual and in multi disciplinary domain.
PO-10	**Communication:** The student is trained in both verbal and written communication in English.
PO-11	**Project management and finance:** Having undergo nea project the student is capable of designing, performing and interpreting there sults of their experiment. There by implement cost effective and improved system
PO-12	**Life-long learning:** Graduate will develop confidence for self education and ability for life-long learning..

Program specific objectives(PSOs)

PSO -1	Biotechnologist will have the basic and advanced understanding of Biotechnology in its various domains includes health, nutrition, agriculture, environmental and Bio safety etc.
PSO -2	Graduates will have the ability to do innovative research in development of eco friendly products and be an entrepreneur.
PSO -3	Graduates will have the ability to work as a member of multidisciplinary teams in various Industrial sectors (Drug development, Forensics, IT, agriculture and Food industry etc.,).

ABSTRACT

The aim of this study is to assess the feasibility of biogas production by implementing the dual goal of developing eco-efficient waste disposal methods and renewable energy y systems by implementing anaerobic co-digestion of flower waste in combination with parsley stem waste. This study received flower waste from Salem floral market and the Parsley stem waste from Flex Foods Ltd, operated in Hosur. The substrates received comprehensive testing for total solids (TS) volatile solids (VS) chemical oxygen demand (COD) and pH together with examination of microbial levels. The experiment ran under

Mesophilic conditions for 21 days according to modified VDI 4630 (2006) guidelines which accommodated available local resources. The researchers utilized a dome setup for simplified gas collection to determine biogas volume through measurements of truncated cone height differences. The collected gas underwent boiling point analysis to provide evidence about combustibility rather than performing direct methane evaluation. The experiments included mono-digestion and co-Digestion procedures. Biogas production through co-digestion exceeded the levels obtained from digesting individual substrates. This analysis validates decentralized biogas systems which incorporate agro-industrial waste together with floral waste for sustainable energy creation and circular waste management solutions. A cost- efficient and efficient method functions for rural areas and small-scale installations.

Keywords:*Biogas,Parsleystemwaste,Flowerwaste,Cowdung,Waste Management*

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LIST OF SYMBOLS AND ABBREVIATION

g	Gram
kg	Kilogram
mg	Milligrams
L or l	Units of volume
ml	Milliliter
M^3	Cubic meter
Nm^3	Normal cubic meter Boiling Point (Combustion Time) of Biogas Samples (BIO1, BIO2, BIO3) over Time
MJ	Mega joule
MJ/m^3	Mega joule per cubic meter
°C	Degrees Celsius
%	Percent
P pm	Parts per million
h	Hour
Cm	Centimeter
Mm	Millimeter
µS/cm	Microsiemens per centimeter
pH	Measure of acidity/base city
L	Volume

1. INTRODUCTION

1.1. RESEARCH BACKGROUND

India is facing two urgent and interconnected challenges an exponentialincreaseinorganicwastegenerationandarisingdemandforcleanandsustainable energy. With rapid urbanization and agricultural expansion, significant quantities of biodegradable waste such as market discards, flower residues, and agro-industrial byproducts arebeingproduceddaily.Theburdenonmunicipalwastemanagementsystemshasresultedin environmentaldegradation,waterandsoilcontamination,andgrowingpublichealthconcerns [1].Simultaneously,theneedforreliableandrenewableenergyalternativeshasbecomecritical to address climate change and ensure long-term energy security.

Anaerobic digestion (AD) presents a viable dual-purpose solution for managing organic waste while generating biogas, a renewable energy source rich in methane. This biological process not only reduces the volume of waste sent to landfills but also provides a clean-burning gas suitable for cooking, lighting, and electricity generation [2]. Among innovative techniques in AD, co-digestion—the simultaneous digestion of multiple substrates—has shown significant promise by improving microbial efficiency and increasing biogas yields through substrate balancing [7].

This project investigates theco-digestion of floralwasteand parsley stemwaste, two largely untapped resources in Tamil Nadu. Floral waste, abundantly available from local marketsandtemplessuchastheSalemFloralMarket,isoftendiscardeduntreated,contributing to environmental pollution. Similarly, parsley stem waste, generated during processing at FLEX FOODS LTD, Hosur, represents a nutrient-rich but underutilized organic stream [9], [10].

A Deenbandhu biogas plant model was selected for this study due to its cost-effectiveness,wideruraladoption,andsuitabilityfordecentralizedenergyproduction[8].The digester was inoculated with 100 kg of fresh cow dung, serving as a microbial starter, and operated under mesophilic conditions for a 21-day period. Mono-digestion of each substrate was carried out separately for 10 days, while co-digestion trials were performed for 21 days.

Thevolumeofbiogasproducedwasestimatedusingatruncatedconevolumeformula based on dome height measurements, offering a practical alternative to conventional gas flow meters.Althoughdirect methane content wasnot analysed,boilingpoint testswereconducted to confirm the combustibility of the gas.

This study aims to assess theefficiency of co-digesting floraland parsley stemwaste for renewable energy generation, while also contributing to sustainable waste management practices.Thefindingsareexpectedtosupportlocalizedwaste-to-energystrategiesinruraland peri-urban regions of India, promoting circular economy models and environmental sustainability.

1.2. CHARACTERSTICSOFBIOGAS

Composition of biogas depends upon feed material also. Biogas is about 20% lighterthanairhasanignitiontemperatureinrangeof650to7500^{0}C.Anodorless&colourless gasthatburnswithblueflamesimilartoLPGgas.Itscaloricvalueis20MegaJoules(MJ)/m3 and it usually burns with 60 % efficiency in a conventional biogas stove. This gas is useful as fuel to substitute firewood, cow-dung, petrol, LPG, diesel, & electricity, depending on the natureofthetask,andlocalsupplyconditionsandconstraints.Biogasdigestorsystemsprovides a residue organic waste, after its anaerobic digestion(AD) that has superior nutrient qualities over normal organic fertilizer, as it is in the form of ammonia and can be used as manure. Anaerobic biogas digesters also function as waste disposal systems, particularly for human wastes, and can, therefore, prevent potential sources of environmental contamination and the spreadofpathogensanddiseasecausingbacteria.Biogastechnologyisparticularlyvaluablein agricultural residual treatment of animal excreta and kitchen refuse(residuals).

1.3. PROPERTIESANDBENIFITS OFBIOGAS

Biogasisacolorless,odorless,andflammablegasproducedthrough theanaerobic digestion of organicmatter. Itprimarily consists of methane(CH_4), carbon dioxide(CO_2), and trace amounts of hydrogen sulfide (H_2S), nitrogen (N_2), and moisture.

Table1.PropertiesofBiogas :

Composition	Typically50–70%methane,30–40%CO_2,andminor gases.
CalorificValue	Rangesfrom20to25MJ/m^3,dependingonmethanecontent
IgnitionTemperature	About650–750°C.
FlammabilityLimit	Methaneisflammablebetween5–15%concentrationinair.
Density	Around1.15kg/m^3atstandardtemperatureandpressure.

BenefitsofBiogas :

Renewable Energy Source: Biogas is derived from organic waste materials, making itsustainable and replenishable.

i. **WasteManagement:**Ithelpsreduceenvironmentalwastebyconverting biodegradable materials into energy.
ii. **EmissionReduction:**BiogascombustionemitslessCO_2thanfossilfuels, contributingtolowergreenhousegas emissions.
iii. **EnergySecurity:** Utilizationof localwasteforenergyenhancesregionalenergy independence.
iv. **SoilEnrichment:**Thedigestateisavaluablebyproductusedasorganicfertilizer, improving soil fertility.
v. **Economic Viability:**Especially in rural areas, biogas offers a cost-effective and scalableenergysolutionwithpotentialforjobcreationandlocalentrepreneurship.

These properties and advantages make biogas an integral component of sustainable developmentstrategies, especially in developing countries likeIndia whereorganicwaste and energy demands are both high.

1.4. NEEDFORTHISSTUDY

Environmental and public health safeguards are compromised by the improper disposalof floralwastetogether with food processing residues found throughoutvarious rural regionsandsemi-urbanareasacrossIndia.Whentheseorganicmaterialsreceiveopendumping treatmentitcreatesbothersomesmellswhichalsoattractsdisease-bearinginsectsandproduces waterpollutionfromleachatecontamination.Parsleystemwasteexistsasnaturallydegradable

organicmaterialbutsocietyusuallytreatsitasuselesswastebeforediscardingitwithoutcareful planning. Such approach represents a wasted possibility to obtain beneficial energy from unusedresources.Researchershaveinitiatedworktoexamineandevaluatethedormantenergy value contained in the co-digestion mixture of parsley stems alongside floral scraps with cow dung. A decentralized 240-liter digester Deenbandhu biogas plant with cost-efficient system functions as a practical solution to generate energy from small-scale operations at regional locations. Through this approach communities can enhance their local sustainability by converting both energy and fertilizer from waste which supports circular bioeconomy principles by maximizing waste stream value and decreasing fossil fuel dependence. The system provides both clean energy access and waste-to-wealth opportunities to local communities which enables their empowerment.

1.5. OBJEVTIVES

- Toevaluatethesuitabilityoffloralwasteandparsleystemwasteas substratesfor biogasproductionbycharacterizingtheirkeyphysicochemicalpropertiesrelevant toanaerobicdigestion(e.g.,totalsolids,volatilesolids,chemicaloxygendemand, carbon-to-nitrogen ratio).
- Tocharacterizethefeedstocks(floralwasteandparsleystemwaste)andthefinal digestatefrommono-andco-digestionexperiments basedonparameters suchas totalsolids (TS), volatilesolids (VS), chemicaloxygen demand (COD), and pH.
- To compare the performance of mono-digestion (floral waste and parsley stem wasteindividuallywithcowdung)and co-digestion(floralwaste andparsleystem waste mixture with cow dung) in terms of total biogas yield and, if possible, methane content.
- To monitor the daily biogas generation from the Deenbandhu biogas plant using thetruncatedconevolumedisplacementmethodforbothmono-andco-digestion trials.
- To assess the total mesophilic bacterial count and, if feasible, the methanogenicarchaeal population in the digestate of the co-digested samples (Bio3) at the end of the digestion period.

1.6. SCOPEOFTHE PROJECT

The project analyzes the anaerobic combination digestion of floral waste obtained from Salem Floral Market and parsley stem waste sourced from Flex Foods Ltd., Hosur. The Deenbandhu-typebiogasdigesteroperatedat240litersservedastheexperimentalsetupdueto itswidespreadhouseholduse.Thissetuputilizedcowdungasthestartingmicrobialpopulation. A 10-day trial served as the period for assessing single feedstock performance during mono-digestion because this time frame is optimal for observing initial production patterns with individual substrates. The digester performed co-digestion of mixed feedstocks over a prolonged time frame of 21 days to enable opposing detrimental effects and achieve whole-scalebreakdownofcombinedorganicmatter.Thetruncatedconevolumedisplacementmethod served to measure daily biogas volume exactly. The researchers analyzed the digestive end productsamplesfromsingle-tank andcombined-tanksetupsforTS,VS,COD,pHparameters as well as microorganism count in Bio3 at the trial's completion date to study co-digestion microbiology. The project establishes Biogas potential assessment and basic characterization as key targets but more detailed analyses of biogas composition and complete microbial community study are not part of this current work.

1.7. SIGNIFICANCEOFTHESTUDY

Under anaerobic degradation of organic waste the microbial process generates biogas whichconsistsmainlyofmethane(CH4)andcarbondioxide(CO2)alongwithtracegases.The controlled process of anaerobic digestion serves as a fundamental sustainable waste management solution which specifically benefits the waste management of floral waste and parsley waste streams that exist on a large scale throughout India. The waste reduction accompanied by renewable energy production makes biogas generation a sustainable waste management solution.

Microbiologicalprocessesandtemperatureconditionsdeterminethechemicalbalanceof this biogas production. To maximize energy recovery from waste materials it is beneficial to have biogas with higher methane levels. The production of biogas includes water vapor at 2-7 volume percent together with minor concentrations of nitrogen (N_2), ammonia (NH_3), oxygen (O_2),hydrogen(H_2)andhydrogensulfide(H_2S).Knowledgeaboutbiogascompositionplaysa

criticalroleinbothimprovingfloralandparsleywasteanaerobicdigestionandensuringbiogas can be safely used as renewable energy to support efficient waste valorization in India.

Table2.compositionof biogas.

Compound	Chemicalsymbol	Content(vol%)
Methane	C_2H_6	50–75
Carbondi oxide	CO_2	25–45
Watervapour	H_2O	2– 7
Oxygen	O_2	<2
Nitrogen	N_2	<2
Ammonia	NH_3	<1
Hydrogen	H_2	<1
Hydrogensulphide	H_2S	<1

Biogas methane potential and energetic worth derive from the biochemical waste compositionwhichgoesthroughthedigestionprocess.Actualmethaneproductionyieldsfrom combinedfloralwasteandparsleywastestreamswilldependontheirindividualprotein,lipid, and carbohydrate concentrations. Indian agricultural and horticultural residues serve as a sustainable waste valorization opportunity through anaerobic co-digestion for renewable energy generation because of their easy availability.

Understanding the biochemical composition of floral waste and parsley is crucial for predicting theachievablemethaneyield and optimizing thedigestion process to maximizethe energetic value of the biogas produced.Considering that a standard biogas methane content of around 50 vol% corresponds to an energetic value of 21 MJ/Nm3, efficient co-digestion strategiescancontributesignificantlytoharnessingtheenergypotentialembeddedwithinthese organic wastes in India.

1.8. BIOGASPLANTLAYOUT

The plant used is a dome-shaped, fixed Deenbandhu-type biogas digester witha200-litergasholdingcapacity.Thestructureismadefromlocallyavailablematerials

andoperatesunderambientconditions.Aninitialinoculumof100kgcowdungisusedbefore feeding the test substrates.

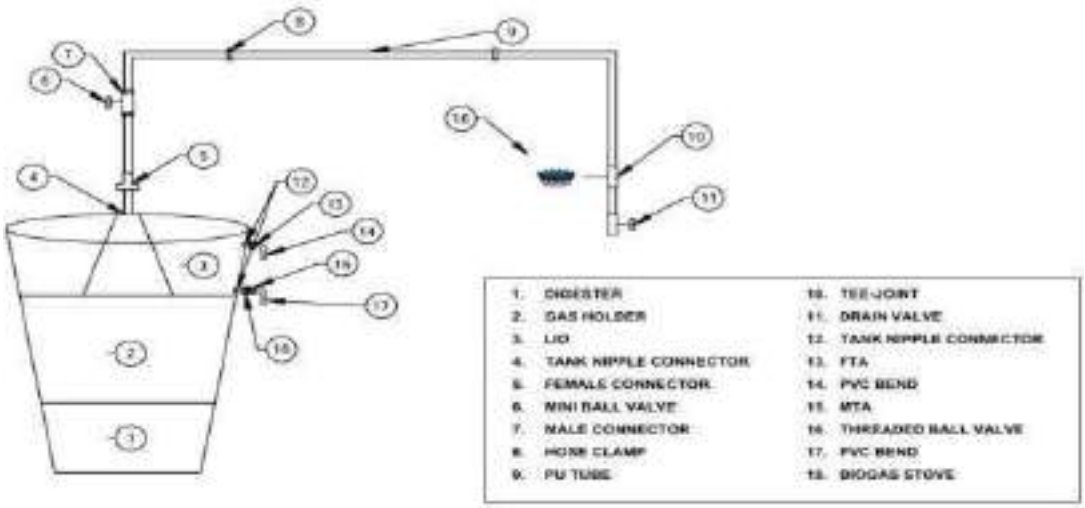

Figure1.biogasplantlayout

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The image depicts a diagram of a biogas plant, likely a small-scale design. The system appearstobeafixed-dometype,wherethedigesterandgasstorageareintegrated.Thediagram labels the various components of the system, showing how they are connected.

KeyComponentsandtheir arrangement.

Digester(1):Thisisthemaincontainerwheretheanaerobicdigestionprocesstakesplace. It's shown as a large, partially buried container.

GasHolder(2):Thiscomponentstoresthebiogasproducedinthedigester.Inthisdesign, it seems to be the upper portion of the digester.

Lid(3):Thissealsthedigester,maintaininganaerobicconditions. Tank

Nipple Connector (4 & 12): Connects parts of the piping.

Female Connector (5): A connector in the piping system.

MiniBallValve(6):Atypeofvalveused tocontrol gasflow. Male

Connector (7): A connector in the piping system.

HoseClamp(8):Securesthehoseconnections.

PUTube(9):A flexiblepipe,probablyforgas transport.

Tee-Joint(10):Aconnector thatallowsthepipetosplitin threedirections. Drain

Valve (11): Used to remove the slurry from the digester.

TankNippleConnector(12):Connectspartsofthepiping. FTA

(13): Likely a type of pipe fitting.

PVCBend(14&17):Acurvedsectionofpipe. MTA

(15): Likely a type of pipe fitting.

ThreadedBallValve(16):Avalvewiththreads,usedtocontrolgasflow. PVC Bend

(17): A curved section of pipe.

BiogasStove(18):Theappliancethatutilizesthebiogasforcooking. Gas

Flow Path:

The diagram shows the flow of biogas. It is produced in the digester (1), accumulates in the gas holder (2), and is then piped through a series of tubes and connectors, passing through valves, to the biogas stove (18), where it can be used for combustion.

1.9. CHALLENGESINBIOGASPRODUCTION

Despite the promising benefits of biogas technology, several critical challenges need to be addressed to ensure consistent and efficient production:

Feedstock Variability: While parsley stem waste is available in large quantities year-round from industrial food processing units like Flex Foods Ltd., floral waste availability is subjecttoseasonalandmarketvariations.ThisinconsistencycanleadtofluctuationsintheC/N ratioand affect digestion performance and biogasyield. Whenfloralwaste islimited,reliance

on alternative feedstocks or adjusting co-digestion ratios becomes necessary to maintain digester stability.

ProcessInhibition:Anaerobicdigestionissensitivetothebuildupofcertaincompounds. The accumulation of volatile fatty acids (VFAs), ammonia, and hydrogen sulfide (H_2S) can inhibit methanogenic bacteria responsible for methane formation. High ammonia concentrations may arise from protein-rich feedstocks, leading to pH imbalance and reduced microbialactivity. Additionally, insufficient micronutrients can hamper microbialgrowth and gas production.

Lack of Awareness and Technical Training: Rural and semi-urban populations often lack access to knowledge and training on biogas plant construction, operation, and maintenance. Misconceptions about the technology and lack of support systems can limit adoption, especially among small-scale farmers.

InfrastructureandInvestmentNeeds:EvenwithlowcostmodelslikeDeenbandhu plant,acertainlevelofinfrastructureisessential.Efficientslurryhandling,regularmaintenance ofgaspipesandvalves,properdigesterinsulation,andsecuregasstoragemechanismsrequire capital investment and skilled manpower. Without these, gas leakage, digester collapse, or operational inefficiencies can arise.

Addressing these challenges requires integrated approaches involving stakeholder awareness, local adaptation of technology, financial incentives, and support through rural development schemes.

1.10. APPLICATIONSOFBIOGAS

Biogas serves as a versatile and sustainable source of energy with a broad spectrum of applications across domestic, agricultural, and industrial sectors:

Cooking: Biogasprovidesasmokeless,soot-freealternativetotraditionalbiomassfuels like firewood and dung cakes. It reduces indoor air pollution and respiratory health risks, particularlyforwomenandchildren.Manyruralhouseholdsusebiogasfordailycookingneeds through simple biogas stoves.

Electricity Generation: Biogas can be combusted in internal combustion engines connected to generators to produce electricity. This is especially beneficial for off-grid rural areas and small-scale industries. Combined heat and power (CHP) units allow simultaneous generation of electricity and heat from biogas, improving overall energy efficiency.

Heating Applications: The thermal energy from biogas combustion can be used for waterheating,spaceheatingingreenhouses,andindustrialheatingprocesses.Thisapplication is common in dairy farms, agro-processing units, and cottage industries.

Transportation: When purified to remove CO_2, H_2S, and moisture, biogas becomes biomethane—afuelcomparableinenergyvaluetonaturalgas.Compressedbiomethane(CBG) canbeusedinvehiclesdesignedforcompressednaturalgas(CNG),offeringacleanfueloption with lower emissions.

FertilizerProduction:Thedigestateremainingafteranaerobicdigestionisnutrient-rich and pathogen-reduced. It contains essential plant nutrients such as nitrogen, phosphorus, and potassium. This bio-slurry can be applied directly to fields or after drying and composting. It improves soil health, reduces dependence on chemical fertilizers, and closes the nutrient loop in agricultural systems.

Overall, the multifaceted applications of biogas contribute to sustainable energy transitions, resource recycling, environmental protection, and improved livelihoods in both rural and urban contexts.

2. LITERATURE RVIEW

2.1. ANAEROBICDIGESTION

Anaerobic digestion (AD) is a biological process in which organic waste is decomposed in the absence of oxygen to create biogas, mostly composed of methane and carbondioxide[12],[23].In thisproject, theADprocesswasapplied toco-digestfloralwaste and parsley stem waste collected from local sources in Tamil Nadu using a 200-liter Deenbandhu-type biogas digester. The system was initiated with 100 kg of fresh cow dung servingasthe microbialinoculum. Unlike simulated models[19], theexperiment inthisstudy did not utilize PROII or AMPTS II systems. Instead, gas production was monitored daily throughatruncatedconevolumecalculationmethod. Methanecontentwasindirectlyassessed via boiling point observation—a simple indicator for combustibility in rural-scale digesters [15], [4].

Co-digestion offers increased methane yields compared to mono-digestion due to the synergistic breakdown of complementary substrates, improving microbial stability and buffering capacity [16], [17], [18]. Studies haveconfirmed thatfood and floralwastes—being richinbiodegradableorganicmatterlikecarbohydrates,proteins,andfibers—areidealforAD systemsandyield significant amountsof methane(50–70%)undercontrolled conditions[20], [21], [23].

Although the experimental design here differs from advanced setups using mathematical simulations or reactor modeling tools [30], [31], it emphasizes a rural, low-cost adaptation strategy that aligns with circular economy principles [22], [24], [9]. Organic waste from markets, agro-processing units, and domestic kitchens, such as floral discards and herb stem residues, continues to be an untapped feedstock in India. Using these materials in Deenbandhu biogas plants represents a practical, sustainable approach to renewable energy generation, waste reduction, and decentralized sanitation [19], [32], [35].

Moreover, previous findings from similar digestion studies highlight the importance of operating parameters—such as organic loading rate, pH, temperature, and C/N ratio—for optimal gas yield [13], [25], [29], [42]. This study reflects these practices by incorporating substrate characterization (TS, VS, COD, pH) and evaluating the final digestate formicrobialpopulationactivity.Bycombiningeasilydegradableparsleystemsand

lignocellulosicfloralresidues,thesystemsustainsgasproductionforextendedperiodswithout system failure [28], [36], [39].

Ultimately, this study supports the notion that small-scale AD using co-digestion of locally available biodegradable wastes can provide a viable solution to India's energy and waste management challenges [29], [33], [39], while promoting environmental sustainability and rural development [26], [34], [43], [47].

2.2. BIOGASFROMANAEROBIC PROCESS

AD leads to the production of biogas along with smaller amounts of other gases throughthefermentationprocess.Aseriesofstagestakesplacesduringtheoverallconversion process. Different species of bacteria usually take place in production of biogas or methane throughaseries ofchemicalreaction.Thestagesofbiogasormethaneproductionisdiscussed here briefly [12], [15], [10], [21], [38].

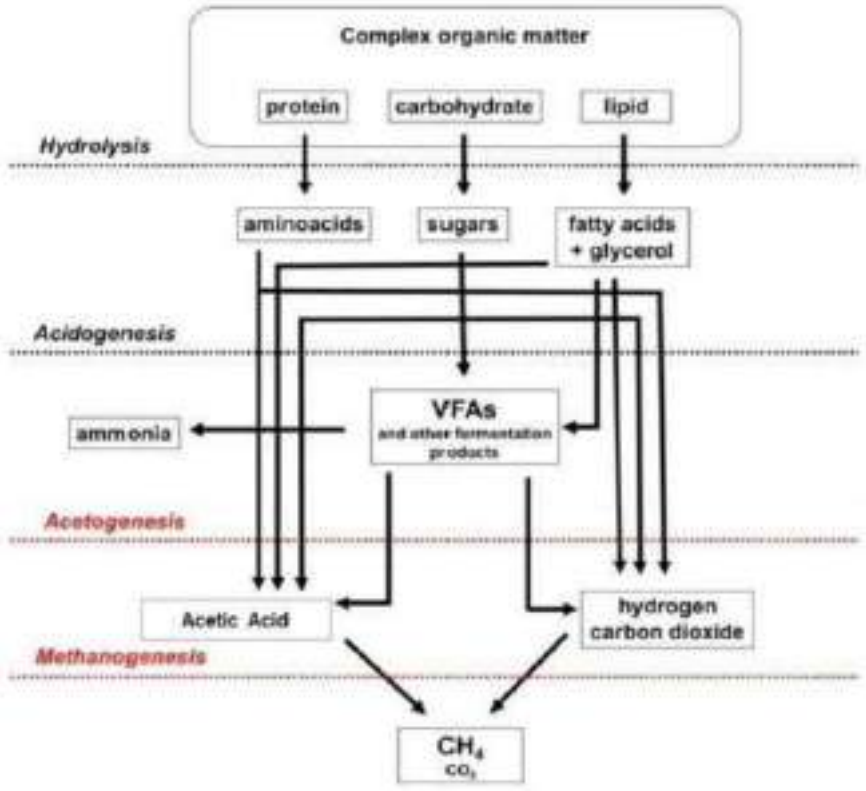

Fig2.Anaerobicdigestionsteps

Thecompletebiogas manufacturingprocedureincludesfouressentialactions.

- hydrolysis,
- acidogenesis,
- acetogenesis,
- methanogenesis.

Complexorganicmaterialundergoesatransformationprocessthatendswiththegenerationof methane as the central biofuel.

2.2.1. Hydrolysis

Hydrolysis is a chemical process through which complex organic substances are decomposed into simpler monomeric units. The reaction below illustrates the hydrolysis of municipalsolidwaste.Thisacid-producingstageinvolvestwoprimarybiochemicalprocesses: fermentation andacetogenesis.Typicalreactionsobservedatthisstageincludetheconversion of glucoseinto ethanoland glucoseinto propionate. Moreover, thetransformations of glucose to acetate, ethanol to acetate, propionate to acetate, and bicarbonate to acetate are all integral to the acetogenesis phase [14], [21], [25], [39].

$$Biomass + H_2O \rightarrow Monomers + H_2$$

Biogas generation was achieved through a 21-day anaerobic digestion process using parsleystemandfloralwastecombinedwithcowdunginoculamtoensurecompletedigestion. This anaerobic digestion process is highly sensitive to pH, with optimal activity occurring within the range of 6.8 to 7.4 [12]. In addition to pH, the temperature of both the digester and the surrounding environment significantly influence the overall efficiency of the process. Duringthe initialtwodays,a slurrywasprepared by mixing fresh cowdungwithtap waterin a 1:5 weight ratio [24], [40].

Thephysicalcharacteristicsofthewaterusedwereassessedbasedonstandardprotocols for water and wastewater analysis. These assessments included total solids, volatile solids, moisture content, and ash content [12], [41]. As global population growth continues to strain limitedresources,thereisanincreasingobligationtoadoptheprinciplesof"reuse,reduce,and recycle" in wastemanagement. Whilesomedegreeof food wasteatIUTmay beunavoidable, the significant environmental impact associated with food waste disposal in landfills can be mitigated. Implementing environmentally friendly technologies, such as anaerobic digestion, can significantly reduce greenhouse gas emissions and support broader sustainability goals.

2.2.2. Acidogenesis

Acidogenic bacteria are responsible for converting soluble organic monomers—primarily sugars and amino acids—into ethanol, various acids (such as propionic and butyric acid), acetate, water, and carbon dioxide. The degradation of amino acids also results in the production of ammonia [26]. These bacteria absorb hydrolysis products through their membranes and generate intermediate volatile fatty acids (VFAs) and other byproducts [42], [43]. VFAs include simple acids like acetate and larger molecules such as propionate and butyrate. Typical composition ratios of these acids range from 75:15:10 to 40:40:20 [44]. Nonetheless, small quantities of ethanol and lactate may still be present.

The concentrations of these intermediate compounds depend on the specific operating conditions of the digester. VFA levels can differ widely between systems functioning under varied pH environments, often leading to inconsistent findings across studies. Acidogenesis generally proceeds at a faster rate than the other anaerobic digestion stages, as acidogenic bacteria can replicate in under 36 hours. However, the rapid accumulation of VFAs is known

to be a major contributor to digester instability and failure. VFAs act as immediate precursors for methane production during the subsequent methanogenesis stage [43], [45], [46]. This biological mechanism shares similarities with Bokashi composting, where microbial action breaks downorganicmaterial.A detailedunderstandingofVFA productionfromaminoacids is especially crucial when managing protein-rich substrates, such as sewage wastewater and amino-containingorganicwastes.Aminoaciddeaminationproducesammonia,whichhasbeen found to inhibit anaerobic digestion at elevated concentrations. During acidogenesis, a range ofcompounds—includingshort-chainvolatileacids(likepropionic,formic,lactic,butyric,and succinicacids),ketones(suchasglycerolandacetone),andalcohols(ethanolandmethanol)— are generated from the breakdown of soluble monomers into simpler organic molecules [13], [47].

$$C_6H_{12}O_6+2H_2\rightarrow 2CH_3CH_2COOH+2H_2O$$

$$C_6H_{12}O_6+ 2H_2\rightarrow 2CH_3CH_2\,OH + 2CO_2$$

2.2.3. Acetogenesis

In the acetogenesis phase, acetogenic bacteria metabolize long-chain fatty acids, volatilefattyacids(VFAs),andalcohols,producing hydrogen,carbondioxide,andaceticacid asbyproducts.ThisbiochemicalconversionplaysasignificantroleinreducingbothBiological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD), while simultaneously loweringthesystem'spH[48]–[50].Hydrogen isakeyintermediate in thisprocess;itspartial pressure must remain sufficiently low to enable the thermodynamically favorable conversion of acids. A decrease in hydrogen partial pressure is thus essential for sustaining this reaction pathway. Accordingly, hydrogen concentration within the digester serves as an indicator of overall system performance and stability.

Despitethetransformation ofsomeVFAs,higher-orderVFAsoftenremaininaccessible to methanogenic microorganisms. Acetogenesis facilitates the further breakdown of these complex intermediates into acetate, concurrently producing hydrogen. This phasehighlights a notablesyntrophicrelationship,wherebytheinterspeciestransferofhydrogensupportsmutual metabolicfunctionwithinthemicrobialcommunityinvolvedinanaerobicdigestion.However, whilehydrogenproductionisanormalaspectofacetogenesis,excessivehydrogen

accumulation—resultinginelevatedpartialpressure—cansignificantlyhinderthegrowthand activity of acetogenic bacteria, particularly acetobacterial strains [12], [13], [49].

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO^{3-} + 3H_2C_6H_{12}O_6 + 2H_2O$$

$$\rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

$$2CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$$

$$HCO^{3-} + 4H_2 + H^+ \rightarrow CH_3\,COO^- + 4H_2O$$

Severalbacteriacontribute toacetogenesis,including:

Syntrophobacterwolinii,propionatedecomposer

Syntrophomonoswolfei,buyratedecomposer

2.2.4. Methanogenesis

Methanogenesis is the final stage in the anaerobic digestion process, where methanogenicarchaeaconverthydrogenandaceticacidintomethane(CH_4)andcarbondioxide (CO_2).Thisstageisinfluencedbykeyreactorparameterssuchastemperature,organicloading rate,andfeedstockcomposition.Biogas,theprincipalproductofthisprocess,ispredominantly composed of methane and carbon dioxide but may also include trace gases such as hydrogen sulfide, nitrogen, oxygen, and hydrogen [52]. A methane content exceeding 45% renders the biogas flammable, with its energy potential directly increasing with CH_4 concentration.

Methanogenic microorganisms are strictly anaerobic archaea. They are extremely oxygen-sensitive;forinstance,99%ofMethanococcusvoltaeandMethanococcusvanniellicells die within ten hours of oxygen exposure [13]–[15]. These microbes utilize a limited range of substrates including acetic acid, methanol, methylamines, formates, and hydrogen. Methanogenesis typically requires higher pH and lower redox potential than earlier anaerobic digestion stages, making in-lab cultivation challenging. Furthermore, methanogenic bacteria regenerate more slowly than other microbes involved in digestion. While typical bacteria survive for 5–16 days, some hydrogenotrophic strains like Methanococcusmaripaludis can double in under two hours. Methanosarcina species are especially robust, capable of withstanding high concentrations of ammonia, salt, and acetate, as well as significant pH fluctuations [51], [54]–[57].

Methanogenesiscontinuesinbatchreactorsuntilbiogasproductionhalts,whichmaytake up to 40 days. Evaluating the content of volatile solids and the sludge's dewatering ability can helpdeterminedigestionefficiency[12]–[14],[28].Commonmethanogenicpathwaysinclude:

$$2CH_3CH_2OH+CO_2 \rightarrow CH_3COOH+CH_4$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

$$CH_3OH \rightarrow CH_4 + H_2O$$

$$CO_2+4H_2 \rightarrow CH_4+2H_2O$$

$$CH_3COO^- + SO_4^{2-} + H^+ \rightarrow 2HCO_3^- + H_2S$$

$$CH_3COO^-+NO^-+H_2O+H^+ \rightarrow 2HCO_3^-+NH_4^+$$

Microorganisms involved in methanogenesis include Methanobacterium, Methanobacillus, Methanococcus, and Methanosarcina, among others [13]. These specialized bacteria,unliketraditionalenzymaticbiofuelproductionsystems,canevenbefoundwithinthe digestive tracts of animals.

Anaerobicdigesterscanutilizediverseorganicsubstratesincludinganimalmanure,food scraps, green waste, plant biomass, and sewage sludge. These materials mainly consist of carbohydrates, proteins, and lipids. However, certain organic components degrade at slower rates, with hydrolysis serving as the rate-limiting step in the decomposition of cellulose and hemicellulose. Recalcitrant substances like lignin, peptidoglycan, and membrane-bound proteins resist natural enzymatic breakdown [13].

Organic waste composition includes moisture, volatile solids, and mineral-rich fixed solids (ash). Volatile solids may be biodegradable or non-biodegradable depending on the material [20], [28]. Pretreatment of biomass enhances anaerobic digestion efficiency by reducing structural resistance, particularly in cellulose and hemicellulose, thereby improving thehydrolysisphase[58].Pretreatmenttechniquesdiscussedinrecentstudiesincludeacidand alkalinetreatments,steamexplosion,andmechanicalsizereduction[59].Commonalkaline

agents used include sodium hydroxide (NaOH), calcium hydroxide ($Ca(OH)_2$), and ammonia (NH_3).

Theoretical methane yield (YCH_4, m^3 CH_4 at STP per kg substrate converted) [20], [60] of a substrate can be calculated based on its elemental composition ($C_cH_hOxN_nS_s$) using the following equation:

$$Y_{CH_4} = \frac{22.4\left(\frac{C}{2} + \frac{h}{8} - \frac{x}{4} - \frac{3n}{8} - \frac{s}{4}\right)}{12c + h + 16x + 14n + 16s}$$

A Deenabandhu biogas system incorporating 100 kg of cow dung as an initial grain to process floral waste together with parsley will use microbial synergy to raise the amount of harvested biogas. The usage of cow dung as an organic inoculation supports the process of hydrolysisandassistsacidogenesiswhileitprovides stabilitytothemethanogenesisstep.This improved process employs the Deenabandhu biogas technology as awidely accepted solution that both improves waste conversion from floral debris and parsley and supports sustainable energy development throughout India. Additional investigations should be conducted with diversemeasurementsoffloralwastetoinitialcowdungratiosandparsleyfordiscovering the most efficient biogas production methodology within the Deenabandhu model.

2.3. Roleof CowDung asInoculum

Cow dung plays a vital role as a microbial inoculum in anaerobic digestion (AD) due toitsrich microbialconsortia,highbufferingcapacity,andnutrientcontent.Inthisstudy,cow dungwasusedastheinitialinoculum—100kgoffreshdungwasloadedinto theDeenbandhu biogas digester prior to introducing the floral waste and parsley stem waste. Its use is critical to initiating and stabilizing the digestion process.

Cow dung is naturally abundant in a diverse population of anaerobic microbes, includinghydrolytic,acidogenic,acetogenic,and methanogenicbacteria,whichare all essential for biogas production. These microbes facilitate the breakdown of complex organic materials intosimpler molecules, eventually producing methaneand carbon dioxide. Methanogenssuch

as Methanobacterium, Methanosarcina, and Methanosaeta found in cow dung significantly contribute to methane yield [61].

Additionally, cow dung provides a balanced carbon-to-nitrogen (C/N) ratio, which helps stabilize the digestion of co-substrates like floral and parsley wastes that may otherwise have nutrient imbalances. Its natural buffering capacity prevents drastic pH drops caused by theaccumulationofvolatilefattyacidsduringtheacidogenesisphase,thusmaintainingoptimal conditions for methanogenesis [62].

Cowdungalso aidsin reducing thestart-up lag phasein newdigesters,ensuring faster microbial adaptation and quicker biogas production. According to Verma (2002), [11]. digestersseeded with cowdungreachstablemethanegenerationfasterthanunseededsystems or those using less complex inocula [11].

InruralIndian contexts,cowdungisan accessibleandcost-effectiveresource,making it particularly suited for small-scale or decentralized digesters like the Deenbandhu model. It supportsthelocalcirculareconomybytransforminglivestockwasteintovaluableenergywhile aiding in the management of additional biodegradable wastes.

Thus, in this study, cow dung not only served as an inoculum but also played a stabilizing and performance-enhancing role in the co-digestion process involving floral and parsley stem waste.

2.4. OPERATIONALPARAMETERS

Efficient anaerobic digestion (AD) relies heavily on maintaining optimal operational conditions that support microbial activity and biogas yield. In this study, specific attention was paid to temperature, pH, C:N ratio, and inoculum management, whichdirectlyinfluencebiogasproductionduringtheco-digestionoffloralandparsley stem waste.

2.4.1. Temperature

The Deenbandhu-type digester used in this project operated under ambient temperature conditions, averaging between 28°C and 37°C, favoring mesophilic bacteria,whichthriveinthe30–40°Crange[12],[13].Thesemicroorganismsaremore resilienttoenvironmentalfluctuationsandrequirelessenergyinputcomparedto

thermophilic communities. Although thermophilic digestion (50–60°C) offers faster degradation and higher gas yields, it is energy-intensive and less feasible for rural or small-scale systems like ours [14]. Hence, the system relied on natural mesophilic digestion, balancing energy efficiency with consistent microbial activity.

2.4.2. pH

The initial inoculum (cow dung) helped stabilize the pH within the optimal range of 6.5 to 7.5, suitable for both acidogenic and methanogenic stages of AD [15]. Duringtheexperiment,pHlevelswereregularlymonitored.Ifthedigestershowedsigns of acidification due to volatile fatty acid (VFA) accumulation, a buffer solution of 15 kg of limestone mixed with 200 L water was added, successfully neutralizing acidity within3–4days.Thisapproach alignswithestablishedpracticeswherelimeorsodium bicarbonate is used to adjust pH levels [17]. The buffering effect of cow dung helped avoid process inhibition and supported a balanced microbial ecosystem.

2.4.3. Carbon-to-NitrogenRatio(C:N)

The C:N ratio is a critical factor in biogas production. Floral waste has a moderate C content, while parsley stem waste contains both nitrogen and fibrous material. Based on literature, an optimal C:N ratio of 20–30:1 supports stable methanogenesis [23]. The co-digestion of floral waste and parsley stem waste in a 1:1 ratio (250 g each) was chosen to balance the carbon and nitrogen content, improving substrate synergy and biogas yield. Excess nitrogen may lead to ammonia accumulation, which inhibits methanogenic bacteria if not properly managed [29].

2.4.4. InoculationandStart-Up

To ensure microbial stability and rapid initiation of the digestion process, 100 kg of fresh cow dung was used as the inoculum prior to sample addition. Cow dung provides a rich microbialpopulation—includinghydrolytic,acidogenic,andmethanogenicbacteria—essential for initiating biogas production [11]. Thefeedstock was added gradually during thefirstweek toallowmicrobialacclimatizationandpreventoverload,whichisknowntocauseacidification

and reactor instability [62]. This strategy enhanced the buffer capacity of the digester and ensuredthatmethane-producingmicrobesthrived,transitioningfrominitialCO_2-dominantgas to CH_4-rich biogas by the end of the first week.

2.5. Importanceof Co-Digestion

Co-digestionistheprocessofanaerobicallydigestingamixtureoftwoormoredifferent organic wastes in the same reactor. This method has emerged as a practical and efficient strategyforimprovingthestability,efficiency,andbiogasyieldofanaerobicdigestionsystems, especially when compared to the mono-digestion of a single substrate.

One of the primary advantages of co-digestion is the synergistic effect achieved by combining substrates with complementary characteristics. For instance, floral waste typically has a high carbon content but is low in nitrogen, while parsley stem waste contributes a more balanced C/N ratio. When co-digested, these feedstocks help maintain an optimal C/N ratio (generallybetween20:1and30:1),whichisessentialforrobustmicrobialactivityandmethane production [64]. Co-digestion also helps dilute toxic compounds present in individual substrates, such as essential oils in flowers or phenolics, which can inhibit methanogenic bacteria if not balanced correctly [61].

Studies by Mata-Alvarez et al. (2011) demonstrated thatco-digestion enhances biogas yield and process buffering capacity, reduces the risk of acidification, and improves the biodegradation of complex substrates [89]. Furthermore, it helps stabilize pH levels, improve nutrient availability, and support a more diverse microbial community, which collectively contribute to better overall digestion performance [65].

Inruralandsemi-urbanareaslikethoseinvolvedinthisstudy,co-digestionoffersadded practical benefits. It allows for the use of locally available and seasonally variable organic wastes—such as floral waste from markets and parsley stems from food processing industries likeFlexFoodsLtd.Theflexibilityofthisapproachensurescontinuityoffeedstocksupplyand enhances the sustainability of biogas systems [66].

Additionally, co-digestion can improve the quality of the digestate, the by-product of anaerobic digestion. A well-balanced digestate has better nutrient content and lower phytotoxicity, making it more suitable as an organic fertilizer [67].

Thus, the co-digestion of floral waste and parsley stem waste not only enhances the biogasyieldandstabilityoftheanaerobicdigestionprocessbutalsosupportssustainablewaste management practices and renewable energy generation in the Indian context.

2.6. POTENTIALSUBSTRATESFORBIOGAS PRODUCTION

Different biodegradable organic wastes produce biogas including agricultural residues andfoodwasteandflowerswaste.Theselectionofspecificsubstratematerialsdetermineshow efficientlyanaerobicdigestionoccursbecauseitinfluencesbothmicrobialpopulationsandgas production levels. Anaerobic digestion behaves more slowly with substrates containing lignin especially when processing wood materials[70]. Error rates from biogas production become minimized when using "floral waste and parsley stem waste" which demonstrate superior degradation properties.

Multiple research investigations have showcased floral waste as a suitable feedstock for producing biogas. The study conducted by Kulkarni&Ghanegaonkar (2019) [71] showed that different techniques enable floral waste to reach higher methane production levels during biogas generation. The study by [72] demonstrated that vegetable and flower waste undergo successfulanaerobicdigestionwhichmakesthemapplicableforrenewableenergyproduction.
Using floral waste together with parsley stems improves process stability by controlling the carbon-to-nitrogen ratio which helps sustain microbial function and produces additional methane.

Grocery waste demonstrates strong potential for biogas production based on research conductedby[101]whoconfirmedtheflowerwastedigestibilitymatchestraditionalsubstrates. The co-digestion process of agricultural waste has been proven important according to [74] because it enhances biogas production rates through effective use of floral waste and parsley stems as sustainable energy sources.

Substrate	**Biogasyield(l/kg-VS)**	**AverageCH_4content(%)**
Cattleslurry	200 – 500	60
Pigslurry	300 – 700	60 - 70

Cow dungslurry	200 – 500	60
Floralwaste	380 – 650	55 - 65
Greenvegetablewaste	400 – 500	55 - 65

Table 3: Biogas yield and average methane content of different organic substrates [75]. VS stands for volatile solids content.

Biogas optimization through plant-based materials demands specific pre-treatment alongside co-digestion strategies according to [71], [76], [77]. Biogas production reaches higherlevelsbecausefloralwasteandparsleystemstogethermaintainbalancednutrientsinthe processwhichstopsinhibitionsandadvancesmethaneoutput.Theinvestigativeprojectselects floral waste and parsley stem waste as main inputs to create an efficient biogas generating system with dual benefits of waste reutilization and renewable energy production.

2.6.1. Co-DigestionofFloralWaste withParsleyStemWaste

Theinvestigationexaminesthecombinedbiogasproductiontechniquethatutilizesfloral waste and parsley stem waste co-digestion. The combination of floral waste and parsley stem waste through co-digestion produces better conditions for anaerobic digestion by maintaining stability of the process while also optimizing C/N ratios and enhancing methane output. Combined management of floral waste with parsley stems represents an environmentally friendly waste strategy that both cuts down on discarded materials and maximizes the use of organic materials [71].

Floral waste accumulates in temples along with markets and events while parsley stem waste mainly originates from food production sites and agricultural areas. The two substrates possessexcellentbiodegradabilitytogetherwithreadilyavailablenutrientswhichqualifythem foranaerobicdigestionprocesses.Theprocessstabilitydependsonmanagingvariationsamong substrates as well as controlling the lignocellulosic composition and inhibitory compounds identified by [72].

The methane yield from floral waste between 250–600 l/kg-VS and parsley stem waste at 300–700 l/kg-VS has been reported along with variables like substrate composition and process conditions [101], [74]. Tests demonstrate that methane production achieves greater levelsthroughco-digestionoperationswhentreatingdifferentwastematerialsindividually.The

biogas yield increases because of combined effects between substrate hydrolysis and higher microbial activities [71]

Theefficiencyofprocessworkdependsstronglyonusingproperratiosofsubstrates.The combinationoffloralwastewhichspeedsupmicrobialactivitiesthroughitscarbohydrate-rich content and parsley stems that establish structural equilibrium to stop acidification and ammonia accumulation makes a potent bacterial environment. Due to existing obstacles different measures need implementation.

Mechanical problems together with microbial activity inhibition arise from impurities such as plastic metal synthetic floral decorations within the system. The treatment method of manualsortingandwashingeliminatesspecifiedcontaminantsaccordingto[69].Thedigestion process depends on both retention time duration together with temperature maintenance conditions.Stablemicrobialactivityfunctionsbestatmesophilictemperaturesnear35degrees Celsius yet thermophilic temperature zone at 55 degrees Celsius usually produces greater biogas output [70].

ThemicrobialstabilityremainsunstablewhenpHchangesbecauseofacidiccompounds generated through floral waste decay. To keep optimal pH levels in digestion systems human operators should monitor theconditions and apply water-based mixtures containing limestone alkaline buffers[68].

2.6.1.1. EnvironmentalandWasteManagement Considerations

Allaspectsrelatedtoenvironmentalimpactsoffloralwastecollectionsystemsincluding agriculturalwasteneedthorough evaluation. Thecombinationofproperwasteseparationwith subsequent composting of non-digestible waste materials together with localized waste treatmentmethodshelpslowercarbonemissions.Theinclusionofwastevalorizationpractices with anaerobic digestion facilitates better sustainability according to [74].

Theresearch investigatesoptimal methodsforcombiningfloralwaste with parsley stem waste during co-digestion to improve biogas outputs while developing circular waste management systems.

2.7. Factorsinfluencinganaerobicdigestion

The multi-stage anaerobic digestion process operates under the influence of multiple technical and biochemical elements. Letterbox Magazine discuses process failure sources including inadequate mixing combined with improper particle size arrangements as well as excessivelyviscoussubstrates[68].Theoccurrenceofimpuritiessuchasplastics,metal,soap, detergent, citrus fruitsand glass in organic waste causes two detrimental effects: both mechanical destruction and microbial suppression [69]. The efficiency of the process is determinedsignificantlybybothtemperaturevariationsandretentiontimeperiod [68].Biogas production together with digestate formation in a plant depends heavily on the physical and chemical environment maintained in the facility. Process stability becomes unstable when the pHdeclinesbecauseofvolatilefattyacidaccumulationcombinedwithammoniaandhydrogen sulfide (H_2S) toxicity along with nutrient imbalances and inhibitory compound presence [68]. Stableanaerobicdigestiondependsonthebalancedgrowthofacidogenicmicrobialpopulations and methanogenic microbial populations as per [68].

Effective biogas production together with system efficiency for floral waste and parsleystemwasteco-digestionrequirespropersubstratecompositionmanagementalongwith temperaturecontrolatmesophilicorthermophiliclevelsandstrictinhibitoryfactor regulation.

2.8. PERFORMANCEANDOPTIMIZATIONMEASURESOFANAEROBIC DIGESTION

System efficiency and stability improvements are supported by implementing specificoperationalpracticesandtechnicalmeasures.AcontinuoussystemcheckmonitorspH values to keep microbial environments in their most active state. The system's efficiency remains stable when excessive feeding is avoided because it helps prevent acidification processes. When acid leads to a pH drop we add a mixture of limestone and water solution containing 15 kg with 200 liters of water to restore pH balance within 3 - 4 days. The system operates at mesophilic temperatures because this optimizes both microbial function and gas production.Thefacilitysuperviseswastecompositiontomanagefloralwasteandparsleystem waste for maintained carbon-to-nitrogen (C/N) ratios during the digestion process.

2.9. BIOGAS ANDDIGESTATEUTILISATION

The combination of biogas production from floral waste and parsley stem waste establishesaneco-friendlysolutiontomanagewastewhileproducingrenewablecleanenergy. The200-litergascapacitytankinourbiogasplanteliminatestherequirementforsophisticated bioreactors. Effective management practices for biogas and digestate enable the complete exploitation of these anaerobic digestion end-products.

Biogas Utilisation :Most applications of biogas require desulfurization followed by drying operations as this process stops metal corrosion while making energy systems more efficient. H_2S needs to be eliminated from the gas stream below 250 ppm via biological desulfurization methods [72]. The process of biogas drying happens naturally because water vapor turns into liquid form as the gas travels through pipework equipment which is cooler [75].

A facility that produces biogas enables three different forms of use including direct combustionandelectricitygenerationandcombinedheatandpower(CHP)systems.Chpunits operated with gas engines reach efficiency levels thatexceed **43%* according to [70].The efficiency of biogas systems is improved by researching micro gas turbines and fuel cells according to [75]. Biomethane obtained from biogas faces restricted market potential because of cost-intensive requirements and infrastructural complications during conversion for public grid distribution and vehicle fuel applications [78].

DigestateUtilisation:Digestatefunctionsasorganicfertilizerinagriculturalusesbecause it contains high nutritional values. Changing digestion process temperature and retention time affects the pathogen inactivation along with the nutrient mineralization and odor reduction capabilities [70].

Theseparationsprocessdividesdigestateintotwofractionswhichincludesolidmaterials andfluidsubstances.Thesolidportionofdigestatefunctionsasanexcellentsoilenhancerafter repurposing it into proper compost and the liquid segment needs treatment in wastewater treatment facilities [78]. The high level of organic material in floral and parsley waste leads digestatefrom our system to serve as a sustainable replacement for synthetic fertilizers thus implementing bioeconomy circularity principles

Theprojectachievesasustainablecombinationofbiogasanddigestateusetoestablish a financially reasonable and environmentally beneficial method to produce biogas from floral waste and parsley stems.

2.10. FUTUREPERSPECTIVESOFBIOGAS PRODUCTION

Anaerobic digestion for biogas creation delivers various advantages that combine environmental conservation with waste elimination and power generation and agricultural benefits. The combined digestion of floral waste with parsley stems shows great potential to convert organic materials effectively into renewable energy systems.

The worldwide adoption of biogas technology grows stronger because global communities now seek fossil fuel-independent renewable energy solutions [70]. To achieve maximum efficiency in biogas production plants additional improvements must be made to both waste management practices as well as process optimization and infrastructure developments.

The steady supply of suitable feedstock must be ensured for biogas plants to work economicallyandreliably.Thebiogasproductionyieldsincreasesubstantially whenscientists expand substrate options while refining their pre-treatment methods [78]. Future feedstock possibilitiesforbiogasproductioncomprisefoodprocessingwaste,agriculturalwastematerials and biodegradable residues from different industrial processes [79].

Real-time monitoring alongside process control innovations and microbial population analysis systems maintain system stability to enhance methane production [79]. The optimization of digestion conditions and failure prevention in the digestion process is made possible by advanced microbial community profiling techniques [70].

Biogas production requires an evaluation of its social and economical effects beyond technological progress. The implementation of biogas systems that use market and food processing floral waste creates dual benefits of local resource sustainability and new employment opportunities within waste management and renewable energy fields [78] . The combination of these techniques allows biogas production from floral and parsley stem waste to become an important element for sustainable energy development together with environmental conservation.

2.11. GAPS IN RESEARCH

Although anaerobic digestion (AD) has been widely studied as a renewable energy technology for managing organic waste, significant research gaps remain, especially in the Indian context where region-specific waste types are abundant but underutilized [13], [12]. Floral waste and parsley stem waste are two such substrates with considerable potential for biogas production, yet their biochemical characteristics and digestibility profiles remain sparsely documented in academic literature [3], [4]. While floral waste has been examined to someextentforitsbiogasyield,studiesthatexploreitsco-digestionwithparsleystemwaste— a food processing industry byproduct—are lacking [10], [6].

Most studies on co-digestion have focused on combinations such as food waste with manureorsewagesludge,neglectingagriculturalherbaceousresidueslikeparsleystemswhich are rich in volatile solids and could improve carbon-to-nitrogen (C:N) balance in mixed feedstocks [89]. Additionally, the seasonal and highly variable nature of floral waste necessitates proper pre-treatment and stabilization strategies, which are often overlooked in small-scale setups [90], [14].

Moreover,despite thewidespreaduseofDeenbandhu-typedigestersinruralIndia,there is insufficient field-scale research evaluating their performance with non-traditional substrate combinations. The majority of existing studies rely on lab-scale reactors with advanced instrumentation (e.g., AMPTS II or liquid displacement units) [91]. However, rural biogas initiatives often employ manual measurement methods such as the truncated cone volume estimation, which are scarcely validated in published research [92], [93].

Another major gap lies in microbial characterization of digestate from such novel substrate combinations. Microbial community profiling is crucial for understanding the synergisticorantagonisticinteractionsduringco-digestion,yetmostrural-scalebiogasstudies omitmicrobiologicalanalysis dueto costor lack of access to molecular tools [94], [95]. Even fewer studies examine digestate quality in terms of its nutrient content, pH buffering, or suitability as a fertilizer, especially when mixed feedstocks are involved [96].

Furthermore,whilecowdungisuniversallyusedas astart-upinoculuminIndianbiogas plants,itsbufferingcapacityandmicrobial richnessinsupportingparsleystem orfloral waste

digestion is not well understood [97]. Also, few long-term studies exist that monitor the stability, gas quality (e.g., methane content), and system resilience when such high-carbon feedstocksareco-digestedcontinuouslyunderfluctuatingenvironmentalconditions[98],[99].

Lastly, there is a gap in life cycle or techno-economic assessments for these types of decentralized, low-cost biogas systems using floral and parsley wastes. A robust cost-benefit analysisorenvironmentalimpactreviewisseldomincluded,leavingstakeholdersunsureabout scaling up such initiatives [100].

3. MATERIALSANDMETHODS

3.1. FEEDSTOCKPREPARATION

The analysis studied biogas production feasibility for floral waste together with parsley stem waste when they undergo anaerobic co-digestion process. The research obtained floral waste and parsley stem waste samples at their different locations in Tamil Nadu India during March 2025. FLEX FOOD LTD based in Devagaapalli within DenkanikottaiTaluk of Krishnagiri District near Hosur provided parsley waste while the floral waste originated from Salem Floral Market which is situated near the Old Bus Stand in Salem. Processing was completedforbothwastetypesbeforetheyentereda200-litergascapacitytankwhichoperated without complex bioreactors. This co-digestion system served as a method to evaluate the combined biogas production and process steadiness by using locally accessible waste from agro-industry.

3.1.1. COLLECTIONOFFEEDSTOCK

3.1.1.1. ParsleyStemWastefromFLEXFOODSLtd., Hosur

Devaganapalli-based FLEX FOODS Ltd. functions as a Tamil Nadu-based major exporter and processor of freeze-dried fruits and vegetables and herbs from its base in DenkanikottaiTaluk, Krishnagiri District. Founded in the 1990s the company maintains its worldwidereputationthroughadvancedfreeze-dryingtechnologyalongwithstrongdedication tointernationalfoodstandards.Thefacilitymaintainshygienicoperationsalongwitheco-

friendly principles and it prioritizes value addition throughout its processes while minimizing waste outputs.

The processing system at the facility processes parsley herb material at industrial scalesduringnormalproductionactivities.Thecompanyharvestsparsleyleavesaftercleaning them before freeze-drying them for European market export. During preprocessing parsley stems get removed to generate agro-industrial waste because they cannot appear in the final parsley product due to both structural and visual standards. The production of daily parsley stem output reaches sizably high levels even though regulators classify it as non-edible biomass.

Thecollection ofparsleystemwaste tookplaceat FLEX FOODSduring leafseparation andcleaningoperationsdirectlyfromtheproductionline.Theresearcherstransportedthestems rightawaytocleancontainerstoblockmicrobialdeterioration.Theanaerobicdigestionprocess required mechanical grinding of stems to minimize their size and increase accessibility for microbial usage.

Thewastecontainsabundantcelluloseandhemicellulosethatmakesitanappropriateco-substrate when combined with floral waste for biogas production. Waste was handled under typical conditions by using raw material which needed no thermal or chemical treatments.

3.1.1.2. FloralWastefromSalemOldBus StandFloralMarket

TheSalemOldBusStandFloralMarketfunctionsasamajorwholesaleandretailflower marketplace which meets both regional and district-level market requirements from Salem, Tamil Nadu. The wholesale distribution hub has been servicing the market for many decades asitsuppliesprimaryflowersincludingmarigold,rose,jasmine,chrysanthemumandlotus.The market sells tremendous flower quantities each day to fulfill needs for religious services and special occasions and business sector decorative purposes.

Flowersstayperishablesoasignificantpartofunsoldordiscardedflowermaterialsgets disposed as organic waste. The discarded floral materials comprise wilted flowers as well as overripeblossomsandeveryfragmentofflowersandflowerpartscutduringsortingoperations. Floral derivatives often get discarded at nearby waste containers or open areas which leads to offensive smells, pest infestations and pollutes the environment because of wrong waste handling.

Floralwastecollectiontookplacebeforegarbagedisposalduringearlymorninghoursat vendors and sorting areas. The waste collection featured a combination of petals that were crushed along with both stems and unopened buds that contained significant carbohydrates together with moisture. The specific mixture enables floral waste to function as an ideal biodegradable material suitable for anaerobic digestion processes.

Amanualsortingoperationfollowedfloralwastecollectiontoremovenon-biodegradable materials consisting of plastic threads along with wrappings and rubber bands. After manual sorting of biodegradable waste the grinder processed it to generate greater surface area and achieve better mixing with parsley stems. The material remained untreated with no drying process and researchers used it fresh so volatile solids remained high.

Floral waste is known to be nitrogen-rich and works effectively as a co-substrate with carbon-rich materials like parsley stem waste. This co-digestion approach balances the C:N ratio, enhancing microbial efficiency and promoting stable biogas production.

3.1.1.3. CowDungasInoculum

In this study, fresh cow dung was used as the initial microbial inoculum to facilitate the anaerobic digestion (AD) process. Approximately 100 kg of cow dung was collected from cattlebreedersnear the experimental site.Thecollected cowdung wasdirectly introduced into the 200-liter Deenbandhu-type biogas digester before any substrate feeding commenced.Cow dung is widely recognized for its rich microbial diversity, containing active populations of hydrolytic, acidogenic, acetogenic, and methanogenic microorganisms. These microbes play a crucial role in breaking down complex organic materials into simpler compoundsandsubsequentlyproducingmethaneandcarbondioxide.Duetoitswell-balanced carbon-to-nitrogenratioandhighmicrobialload,cowdungnotonlyactsasastarterculturebut also helps in maintaining process stability, especially in rural-scale digesters.

The cow dung was not subjected to any pretreatment; it was mixed with water in a 1:1 ratio to ensure better slurry consistency and microbial activity distribution. This inoculationphasewasessentialto establishastableanaerobicenvironmentwithin thedigester andtopreventanydelaysinmicrobialadaptationoncethefeedstocks (parsleystemwasteand

floralwaste)wereintroduced.Theuseofcowdungas inoculumalignswith traditionalpractices and supports cost-effective, decentralized waste management strategies, particularly in rural Indian contexts. Its natural buffering ability also assists in maintaining favorable pH levels during the initial days of digestion, minimizing the risk of acidification and process failure.

3.1.2. WASTESORTINGANDCLEANING

Previously parsley stem waste was collected in FLEX FOOD LTD in a clean, uniform, and properly handled condition, so it did not require any additional cleaning or sorting. The company ensured that the stems were free from contaminants and ready for direct use in choppingandslurrypreparation.IncontrastfloralwastewascollectedinSalemOldBusStand Floral Market and they were not sorted. But our aim was to sort the major wastes in proper labels.In Figure3 itisdemonstratedhowwaste wascollectedbefore and Figure4 showshow we have sorted waste in proper labels.

During the initial phase of the project, floral waste collected from the Salem Flower Marketwashandledinanunsortedmanner,whereall typesofwastelikefreshflowers,decayed petals, stems, leaves, plastic covers, synthetic threads, and packaging were indiscriminately dumped into large drums or heaps. This mixed waste, as seen in Figure 3, presented several challenges: it hindered efficient grinding, introduced non-biodegradable contaminants, disruptedslurryuniformity,andposedariskofmicrobialinhibitionduringanaerobicdigestion. To address these issues, a systematic sorting and cleaning process was implemented. In this improved method, floral waste was manually separated into categories based on component type (petals, stems, leaves) and physical condition (fresh, wilted, dried). Non-biodegradable items such as plastic wrappers, cups, threads, and contaminated debris

Fig3.Mixedwastecollection

werecarefullyremovedanddiscarded.Thesortedfloralbiomass,showninFigure4,wasthen washed to eliminate dust, dirt, and possible chemical residues. This cleaned and segregated feedstocknotonlyimprovedvisualandoperationalhygienebutalsoallowedforbettercontrol overfeedstockquality and composition. Theability todistinguish high-moistureflowerpetals from fibrous stems enabled us to customize slurry mixing ratios with parsley stem waste, ensuringbalancedcarbon-to-nitrogen(C:N)ratiosandappropriatetotalsolidscontent.Cleaned biomass was easier to grind, created a more homogeneous slurry, and reduced the risks of floating scum layers or sedimentation in the digester.

Fig4.improvedmethod,floralwastewasmanually separated

Overall,the  wastesortingandcleaning process significantly improved feedstock digestibility,enhanced microbial access, and supported more consistentandefficient biogas production. This intervention also demonstratedtheimportanceofsource-levelwastesegregationinconvertingfloralwastefrom an unmanaged nuisance into a valuable energy resource.

3.1.3. CHOPPINGAND CRUSHING

Following the sorting and cleaning process, the feedstock underwent physical size reduction through chopping and grinding to improve its biodegradability during anaerobic digestion.Inoursetup,parsleystemwaste,beingfibrousandstructurallytough,wasmanually

chopped into small pieces using scissors to facilitate microbial access during digestion, as demonstrated in Figure 5(a). On the other hand, floral waste, composed mostly of soft petals and floral residues, was subjected to wet grinding to convert it into a semi-liquid pulp. The grinding was carried out using a traditional basin setup, where water was gradually added to the floral waste while it was stirred and passed through the outlet below, as demonstrated in Figure5(b). Thisstep helpedhomogenize the mixtureandform aslurrysuitable fordigestion. Theadditionofwaternotonlyhelpedinreducingtheviscosityofthebiomassbutalsoensured thetotalsolidscontentremainedwithintheoptimalrange(8–12%)forwetanaerobicdigestion. Theend resultwas aconsistent, flowablesubstrate wherethechopped parsley stems provided structural balance and the ground floral waste contributed high volatile solids for enhanced methane production. This combined approach ensured that both coarse and soft organic componentswereproperlytreated,ultimatelyimprovingsubstratedegradationandbiogasyield efficiency.

Fig.5(a).ChoppingofparsleystemWaste

Fig.5(b).GrindingofFloralWastewith WaterAdditionforSlurry Preparation

3.1.4. WeightMeasurement

Accurate and consistent measurement of feedstock is essential for controlling the digestionprocessandensuringreliableresultsinbothmono-andco-digestion experiments.In thisstudy,floralwasteandparsleystemwastewereweighedseparatelyusingacalibrated

digitalweighingbalancepriortomixingorprocessing.Thishelpedmaintaintheuniformityof total solids (TS) and ensured consistent organic loading in each trial.

For the co-digestion setup, two different organic wastes were measured in equal proportions—250 grams of floral waste and 250 grams of parsley stem waste, maintaining a 1:1ratiobyweight.Thisratiowaschosentoexplorethesynergisticeffectofcombiningahigh-moisturesubstrate(floralwaste) with afibrous, structured one(parsley stems), thus balancing nutrient availability and biodegradability.

In addition, mono-digestion trials were performed to analyze the individual behavior of eachsubstrate. Forthese,500gramsoffloralwasteand500gramsofparsleystemwastewere measured independently, keeping all other conditions constant. These measurements helped establish a baseline for evaluating gas yield, digestion rate, and stability when substrates are processed separately.

Theimagesbelowdocumentthefeedstockquantities:

Figure6a:250goffloralwasteforco-digestion([InsertImageofFloralWasteonScale])

Figure 6b: 250 g of parsley stem waste for co-digestion ([Insert Image of Parsley Stem Waste on Scale])

Figure 6c: 500 g of floral waste for mono-digestion ([Insert Image of Floral Waste for Mono-digestion])

Figure6d:500gofparsleystemwasteformono-digestion([InsertImageofParsleyStem Waste for Mono-digestion])

By weighing each sample precisely, this method supports clear interpretation of results, accurate mass balance calculations, and the standardization of input material across all digestion experiments.

3.1.5 SlurryMixingandSamplePreparation

After weighing the required quantities of floral waste and parsley stem waste, each samplewasmixedwithwater in a1:1ratio toform a uniformslurry. Forbothmono-digestion and co-digestion trials, this ratio was maintained—500 g of waste to 500 mL of water—to ensure consistent total solids (TS) content, typically ranging between 8% and 12%, which is ideal forwet anaerobic digestion. Floralwaste, being softand moisture-rich,formed asmooth slurry upon grinding, while parsley stem waste, being fibrous, was manually chopped and mixed with water to create a coarser blend.

The slurries were thoroughly stirred using clean utensils to ensure even distribution of solids and water. Separate transparent containers were used for each treatment—mono-digestion with floral waste, mono-digestion with parsley stem waste, and co-digestion with equalpartsofboth.Theseslurrieswerethenlabeledandstoredunderobservationforbuoyancy behavior analysis.

3.1.6 SinkingorFloatingAnalysis

Toevaluatethephysicalbehaviorofthepreparedslurries,afloatationtestwasconducted immediately after mixing. This test aimed to observe whether the biomass would float or sink over time in the slurry a critical factor in digestion efficiency.

Inthecaseofparsleystemwaste,thematerialsankimmediatelyuponmixingwithwater due to its dense and fibrous structure, which readily absorbs water and becomes heavier than the liquid medium [1]. In contrast, the floral waste floated on the surface and remained suspended for approximately 7 to 9 days. This delayed sinking is caused by the hydrophobic waxy cuticle present on flower petals, which repels water and traps air, thereby making the biomass buoyant [3, 4].

Overtime,microbialhydrolysisandenzymaticbreakdownofthefloraltissuesoccurred, softening the structure and allowing water to penetrate. This gradual increase in effective density eventually caused the floral waste to sink by the end of the first week [2]. In the co-digestionmixture,parsleystemssettledquickly,whilepartialsinkingof thefloralportionbegan around Day 3–4.

These observations are visually represented in Figure 7, where the co-digested slurry showssunken parsley stem waste at the bottom and floating floral waste on top during Day 1. Understandingthisbehaviorisessential,asprolongedfloatationmayresult inscumformation, hinder microbial contact, and affect overall gas production efficiency [1, 5].

Fig.7(a).Floatationbehavior of parsley stem waste slurry onDay 1.

Fig.7(b).Floatationbehavior of floral waste slurry on Day1.

Fig.7(c).Settlingoffloral waste by Day 8.

3.2. SUBSTRATE CHARACTERISATION

To assess the biogas potential of the substrates, floral waste (Bio2), parsley stem waste (Bio1), and their co-digestion mixture (Bio3) were characterised for their chemical oxygen demand (COD), total solids (TS), volatile solids (VS), pH, and electrical conductivity (EC).Allmeasurementswereperformedintriplicate,andresultsdeviatingmorethan20%from the mean were considered outliers and excluded.

COD was estimated following the standard protocol described in IS 3025 (Part 58):2006, using aUV-Visible spectrophotometer. The substrates were [mention any dilution or homogenization steps if applicable, similar to your example].

TS and VS were determined according to the protocols described in IS 3025 (Part 15):2006.Totalsolidscontent(TS)wasdeterminedbydryingthesampleat105°Cuntilweight constancy and was expressed in weight percentage according to the formula (1):

$$TS[\%] = \frac{driedsample[g]}{originalsample[g]} x100\%$$

Volatile solids content (VS) was determined by an additional ignition of the dried samplesin amufflefurnaceat550°Cfor twohours. VSwasfirstlyexpressedinpercentageof the TS according to the formula (2) and subsequently in percentage of the total sample according to the formula (3):

$$VS_{(TS)}[\%] = \frac{weightlossonignition[g]}{driedsample[g]} x\ 100\ \%$$

$$VS_{(totalsample)}[\%] = \frac{TS[\%]xVS(TS)[\%]}{100\ \%}$$

Total fixed solids(TFS)were determinedby theresidueremaining afterignition in the muffle furnaceat550°C,followingtheprotocolinIS3025(Part16):[Specifyanyspecificequipment or calculation details if available]. TFS was expressed in [Specify units, e.g., weight percentage] according to the formula (4):

$$TFS[\%] = 100\% - VS_{(totalsample)}[\%]$$

pH was measured using a calibrated pH meter according to IS 3025 (Part 11): 2022, andTemperaturewasmeasuredusingacalibrateddigitalthermometerwithstainless-steelprobe according toIS13464:1992(Reaffirmed 2018).Theseparametersareessential to evaluate the ionic strength and stability of the digestate matrix.

3.3. GAS PRODUCTION

The biogas production experiment was conducted for 21 days under anaerobic conditions. Instead of a standard liquid displacement method, the volume of biogas generated was estimated by measuring the height difference and diameter of the gas collection dome.

3.3.1. Experimentalsetup

The biogas production experiment consisted of three experimental lines, each conducted in triplicate, to evaluate the performance of mono-digestion and co-digestion of parsleystemwasteandfloralwasteusingcowdungastheinoculumorcatalyst.Allsetupswere operatedunderidenticalenvironmentalconditions,andgasproductionwasmeasuredevery24 hours using the truncated cone formula.The experimental lines were as follows:

Bio1:Mono-digestionofParsleyStem Waste(PSW+CD)

→cowdung(100kginitialinoculum)+Parsleystemwaste(500g/day) +2 Lofwater Bio2:

Mono-digestion of Floral Waste (FW+CD)

→cowdung(100kginitialinoculum)+Floralwaste (500g/day)+2Lofwater Bio3: Co-

digestion of Parsley Stem Waste and Floral Waste (PSW+FW+CD)

→ cow dung (100 kg initial inoculum) + Parsley stem waste (250 g/day) + floral waste (250 g/day) + 2 L of water

Eachlinewasmonitoreddailyforbiogasproduction.Mono-digestionlines(Bio1and Bio2) were analyzed over a 10-day period, while co-digestion (Bio3) was conducted over 21 days to observe synergistic effects and stability of biogas output. Methane content was not measuredinthisstudy;totalgasvolumewasusedasthekeyindicatorofsubstrateperformance.

3.3.2. GasPotentialMeasurementwithTruncatedConeFormula

In this project instead of employing a standard liquid displacement method, as often recommended in guidelines like VDI 4630 (which is a relevant VDI guideline for anaerobic digestion),thisstudyutilizedamethodbasedon thegeometryof thegascollectiondome. The volume of biogas generated during the experiment was estimated by measuring the height differenceanddiameterofthisdome.Thisalternativeapproachwaslikelychosentosuitthe

specific experimental setup and available resources, representing a practical adaptation of standardmethodologies.Theunderlyingprincipleisthedirectmeasurementoftheaccumulated biogas volume within a defined geometric space.

Thevolumewasthencalculatedusingthetruncatedconevolumeformula:

$$V=\frac{\pi h}{3}(r_1^2+r_1r_2+r_2^2)$$

Where:

V =Volumeof biogas

h = Height difference of the gas dome (the change in height as the gas accumulates over a specific time interval)

$r_1$andr_2=Radiiofthetopandbottomcirclesofthetruncatedconeshapeassumedfor the gas collection dome. These would have been measured based on the physical dimensions of the dome used in the experimental setup.

This method provided a simplified yet effective means of estimating gas production, particularly given the constraints of available equipment. While VDI guidelines often detail specific requirements for gas measurement accuracy, this adaptation offered a feasible way to track biogas generation over the 21-day experimental period. The measurements were taken every24hoursfor eachof the three experimental lines,providing atemporalprofileof biogas production for the mono-digestion and co-digestion processes.

3.3.3. MethanePotentialMeasurement

Standard methane potential tests, often conducted according to guidelines and using systemslikeAMPTSII(asmentionedinKanger,2013),involvethedirectandquantitative measurementofmethanecontentin theproducedbiogas.Thesemethodstypically employgas chromatography or automated systems with CO2 scrubbing to determine the specific volume orpercentageofmethane.However,inthisstudy,adheringtotheprincipleofadaptingstandard guidelines(likeVDI4630)toavailableresources,adirectmethaneanalysiswasnotperformed.

Instead,amorequalitativeapproachwasadoptedtogainanindicationofcombustible gaspresence.Theboilingpointofthecollectedbiogaswasdeterminedasaproxyformethane content. Thismethodrelieson theunderstanding thatmethane,beingtheprimarycombustible componentofbiogas,hasadistinctboilingpoint.Changesintheboilingpointof thecollected gas mixture could suggest variations in the proportion of methane relative to other gases like carbon dioxide. While VDI guidelines would typically recommend direct gas composition analysisforaccuratemethanepotentialdetermination,theboilingpointmeasurementoffereda simpler,albeitlessprecise,methodtoassessthepresenceofcombustiblegasesunderthegiven experimental limitations.

3.3.4. DataValidation

To ensure the reliability of the experimental results, several data validation measures were implemented, aligning with the general principles of good scientific practice and recommendations found in guidelines like VDI 4630.

First,thebiogas productionexperimentwas conductedwiththreeexperimentallines, each run in triplicate. This repetition (n=3 for each condition) is crucial for assessing the reproducibility of the observed biogas yields for the mono-digestion of parsley stem waste, floral waste, and their co-digestion. By having triplicate setups for each condition, it becomes possible to calculate average biogas production values and determine the variability (standard deviation or standard error) within each treatment group. This allows for a more robust statistical analysis and a better understanding of the consistency of the anaerobic digestion process under the tested conditions.

Second, all experimental setups were operated under identical environmental conditions. Maintaining consistent temperature, and other relevant parameters, is essential to minimize the influence of extraneous variables on biogas production. This ensures that any observed differences in biogas yield between the experimental lines can be more confidently attributedtothedifferentsubstratemixturesratherthanvariationsintheoperatingenvironment.

Third, theduration of themonitoring period was tailored to thespecificexperimental lines. The mono-digestion trials (Bio1 and Bio2) were monitored for 10 days, while the co-digestiontrial(Bio3)wasmonitoredforalongerperiodof21days.Thisextendedmonitoring

oftheco-digestionprocesswaslikely intendedto captureanypotentialsynergisticeffects that mightemergeovertimeandtoassessthelong-termstabilityofthebiogasproductionfromthe mixed substrate.

Finally, while the methane content was not directly quantified, the total gas volume, estimated using the truncated cone formula, served as the primary metric for evaluating substrate performance. This quantitative measure of overall biogas production allowed for a comparison of the total gas yield from the different mono-digestion and co-digestion setups, providing insights into the relative efficiency of the different substrate combinations for anaerobic digestion.

Byimplementingthesemeasures,thestudyaimedtoensurethevalidityandreliability ofthecollectedbiogasproductiondata,giventheconstraintsoftheavailableresourcesandthe chosen methodologies

2.4. DIGESTORCHARACTERIZATION

Following the anaerobic digestion process, the digestate was subjected to a series of physico-chemical and microbiological analyses to assess its stability, residual organic content, and potential for reuse or safe disposal. The parameters analysed include Chemical Oxygen Demand (COD), Total Solids (TS), Volatile Solids (VS), Total Fixed Solids (TFS), pH, Temperature and Total Microbial Count.

The analytical procedures were carried out in accordance with the relevant Indian Standards (IS) as listed below:

TotalSolids(TS),TotalFixedSolids(TFS)andVolatileSolids(VS)weredetermined gravimetrically. TS was measured by oven-drying the sample at 105°C until constant weight asperIS3025(Part15):2006.VSwasthenevaluatedbyignitingthedriedresidueinamuffle furnace at 550°C, in accordance with the same standard.

Chemical Oxygen Demand (COD) was estimated using the closed reflux titrimetric method, a standard protocol described in IS 3025 (Part 58): 2006. This analysis helped determine the residual organic load in the digestate.

pH was measured using a calibrated pH meter according to IS 3025 (Part 11): 2022, andTemperaturewasmeasuredusingacalibrateddigitalthermometerwithstainless-steelprobe according toIS13464:1992(Reaffirmed 2018).Theseparametersareessential to evaluate the ionic strength and stability of the digestate matrix.

2.4.1. MicrobialCount Analysis

Total Microbial Count was assessed using the pour plate technique as per IS 5402:2012. The digestate sample was serially diluted and plated on nutrient agar, followed by incubationat37°Cfor24–48hours.Colony-formingunits(CFU/mL)werethenenumeratedto estimate the viable microbial population.To evaluate the microbial activity in the digester outlet,amicrobialcountanalysiswasperformed.Thesampleselectedforthisanalysiswasbio- slurry obtained after the anaerobic co-digestion of parsley stem waste and floral waste. Approximately 200 mL of the bio-slurry sample was collected in a clean, good-quality plastic bottle. The sample was labeled appropriately and submitted to the United Testing and Analytical Laboratory (UTAL), Salem, Tamil Nadu for microbial analysis. The microbial enumeration was carried outfollowing theAmerican PublicHealth Association (APHA) 24th Edition standard methods:

Heterotrophic Plate Count (HPC) was determined according to APHA Part 9215, whichestimatestheviableaerobicbacteriapopulation.YeastandMoldCountwasdetermined according to APHA Part 9610, which quantifies fungi capable of growing in the digestate. Standard plate count methods were adopted, and results were reported in terms of colony-forming units per milliliter (CFU/mL). The analysis was done under controlled laboratory conditions to ensure the reliability and accuracy of the results.

4. RESULTSANDDISCUSSION

4.1. CharacteristicsofTheSubstrate

Parsleystem waste,floral waste,(bio1, bio2)andtheir co-digestedmixture (bio 3) were characterised in terms of total solids content (TS), Total volatile solids (TVS), Total FixedSolids(TFS),chemicaloxygendemand(COD)andpH-valuewasmeasuredtoidentify

potentialinhibitoryfactorsaffectinganaerobicdigestion.Theresults,includingstandard deviations, are presented in Figure 8.(TS and VS), Figure 3 (COD) and Table 6 (pH).

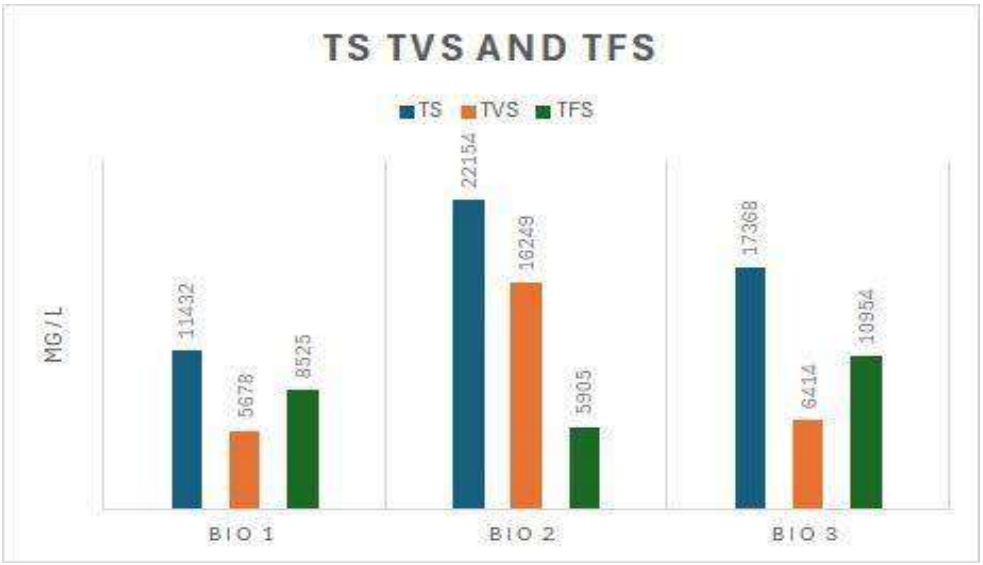

Figure8. Totalsolids(TS)volatile solids(VS) and Total FixedSolids(TFS) content in the substrates. Bars represent standard deviations. Bio1, Bio2 and Bio3 stand for Parsley stem waste, floral waste andtheir co-digested mixture.

Figure9. Chemicaloxygendemand(COD)ofthesubstrates.Barsrepresentstandard deviations.Bio1,Bio2andBio3standforParsleystemwaste,floralwasteandtheirco-digested mixture.

	pH	**Temperature**
Bio1	4.57	32°C

Bio2	6.56	34°C
Bio3	6.87	36°C

Table 4.pH-value of the substrates at the given temperature. Bio1, Bio2 and Bio3 stand for Parsley stem waste, floral waste andtheir co-digested mixture.

The substrate characterization of floral waste (Bio2) and co-digested mixture (Bio3) was conducted prior to digestion to evaluate their suitability for anaerobic biogas production. Theanalysisincludeddeterminationoftotalsolids(TS),volatilesolids(VS),chemicaloxygen demand (COD), pH, and electrical conductivity (EC). Results are summarized in Table X and graphically represented in Figures X–X.

Bio2(floralwaste)showedaTScontentof10.65%,withacorrespondingVSof9.4%, indicating a high organic fraction suitable for microbial degradation. The COD value was measured at 134,210 mg/L, demonstrating a strong potential for biogas generation. The pH valueof6.87waswithintheoptimalrangeforanaerobicdigestion,whiletheECof3.42mS/cm suggested adequate availability of ionic nutrients.

Bio3 (co-digested floral and parsley stem waste) exhibited a TS of 10.97% and a VS of 9.24%. The COD was slightly lower than Bio2 at 132,010 mg/L, yet still indicated a favorable organic load. A pH of 6.81 and EC of 2.8 mS/cm were also within the acceptable operationalrange,indicatingthatthemixtureprovidedastableandnon-inhibitoryenvironment for microbial activity.

These results confirm that both substrates, particularly the co-digestion mixture, are well-suitedforanaerobicdigestionandholdgoodpotentialforbiogasproduction.Bio1(parsley stem waste) was not included in this phase due to observed inconsistencies in data, and its characterization will be presented in the following review phase.

4.2. BIOGASPRODUCTIONANDINDICATIVEMETHANE POTENTIAL

Thetotalvolumeofbiogasproducedoverthe21-dayexperimentalperiodforeach experimentalline,estimatedusingthetruncatedconeformula,isillustratedinFigure.4.Biogas Volume Graph.

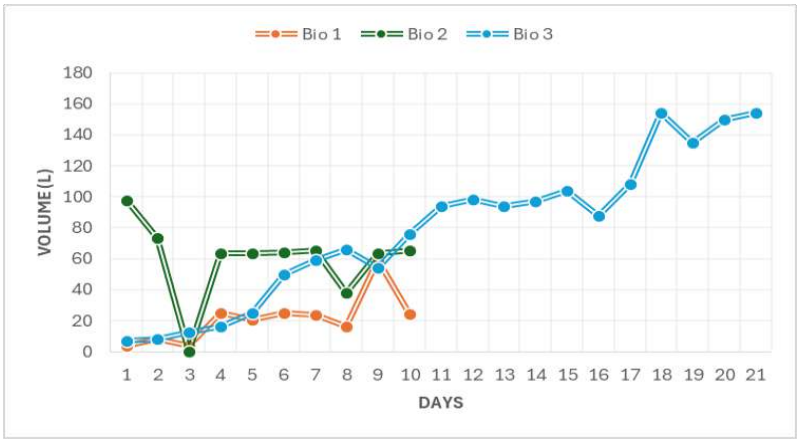

Figure10.Biogas VolumeGraphforbio1,bio2,bio3

The biogas production profiles revealed distinct patterns for each substrate combination. Bio1 (mono-digestion of Parsley Stem Waste) exhibited a gradual increase in biogas production, reaching a peak volume of approximately 59.24 L around day 9. Bio2 (mono-digestion of Floral Waste) showed a more rapid initial biogas production, peaking at roughly 97.77 L on day 1, followed by a sharp decline and subsequent fluctuations, ending at 65.32 L on day 10. Notably, Bio3 (co-digestion of Parsley Stem Waste and Floral Waste) demonstrated a generally increasing trend in biogas production throughout the 21 days, achieving the highest final volume of 154.36 L on day 21, which suggests a synergistic effect of co-digestion on total biogas output over the longer duration.

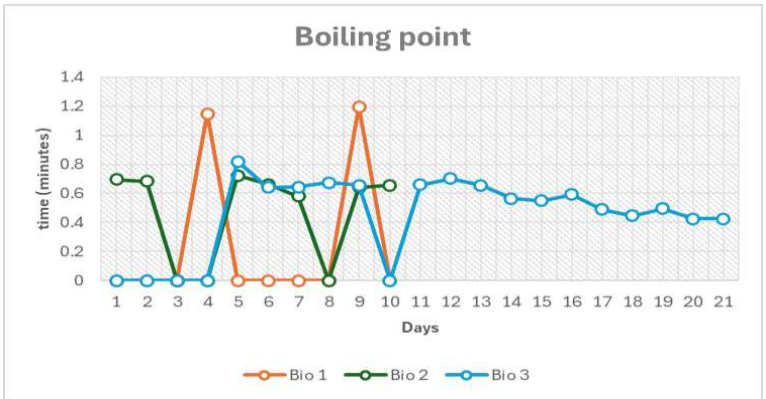

Figure11.boilingpointofthecollectedbiogas

Figure.11. represents the boiling point of the collected biogas, used as an indicative parameter for combustible gas presence. It's important to reiterate that this is not a direct measurement of methane content. Bio1 showed boiling points fluctuating between 0 minutes and 1.15 minutes. Bio2 exhibited a more variable boiling point, with notable peaks early and aroundday8.Incontrast,Bio3generallyshowedafluctuatingbutoverallowertrendinboiling point after an initial rise.

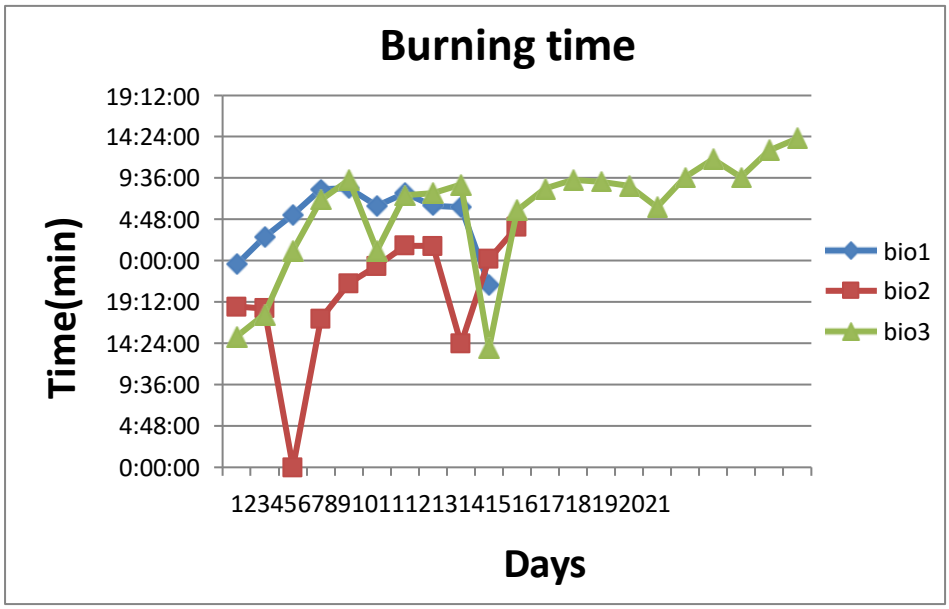

Figure12.boilingpointofthecollectedbiogas

The burning time of the biogas produced from each experimental line is represented in Figure.12. Bio1 exhibited a burning time ranging from approximately 21 minutes to 32 minutes, with some variability across the experimental period. Bio2 showed a more erratic burning time, with periods of no sustained burning observed (0 minutes) and other periods reaching up to 27 minutes. Bio3 generally displayed a longer and more stable burning time, rangingfromapproximately15minutesto38minutes,withatendencytowardslongerburning durations in the later stages of the experiment. Longer burning times in Bio3 suggest a potentially higher concentration of combustible gases, further supporting the inference from theboilingpointdataregardingapossiblyenhancedmethanecontentintheco-digestedbiogas.

However, it is crucial to acknowledge the limitations of using boiling point and burning time as proxies for methane content. While these parameters can provide qualitative indicationsofthebiogas'sflammabilityandpotentialenergycontent,theydonotoffera

quantitativemeasureofmethaneyield.Furtheranalysisusingtechniquessuchasgas chromatography is necessary to accurately determine the methane composition of the biogas.

DAYS	Volume(L)			Burningtime(min)			Boilingpoint(min)		
	Bio1	**Bio2**	**Bio3**	**Bio1**	**Bio2**	**Bio3**	**Bio1**	**Bio2**	**Bio3**
1	4.08	97.77	7.23	23:35:20	18:40:43	15:11:06	0	16:42:45	0
2	8.171	73.21	8.171	26:45:10	18:27:32	17:43:06	0	16:23:21	0
3	4.089	0	12.601	29:20:06	0	25:09:09	0	0	0
4	24.73	63.74	16.486	32:17:17	17:15:23	31:52:44	27:32:11	0	0
5	20.608	63.76	24.73	30:25:22	21:21:23	33:23:33	0	17:21:12	19:43:12
6	24.703	64.12	50.12	30:22:43	23:23:32	25:09:33	0	15:52:32	15:23:21
7	24	65.32	59.26	31:52:32	25:43:51	31:35:22	0	13:54:12	15:25:54
8	16.48	37.83	65.77	30:23:10	25:43:21	31:52:51	0	0	16:12:51
9	59.24	63.21	54.06	30:14:23	14:21:12	32:51:23	28:43:34	15:23:43	15:43:43
10	24.2	65.32	75.85	21:13:21	24:11:11	13:51:43	0	15:43:12	00:00:45
11			93.72		27:54:22	29:51:13			15:49:25
12			98.61			32:23:12			16:51:31
13			94.13			33:22:21			15:43:32
14			96.9			33:12:12			13:33:16
15			103.81			32:41:12			13:12:19
16			87.72			30:13:43			14:13:56
17			108.14			33:41:21			11:42:17
18			154.36			35:49:43			10:43:17
19			134.8			33:41:45			11:51:19
20			149.95			36:49:23			10:10:17
21			154.36			38:12:21			10:11:23

Table.5.Biogas volume,burningtimeand boilingpointforbio1,bio2and bio3.

4.3. DIGESTATE CHARACTERIZATION

The digestate resulting from the anaerobic co-digestion of parsley stem waste and floral waste (Bio3) was subjected to a comprehensive physico-chemical and microbiological characterization to determine its key properties. The analytical methodologies employed are detailed below:

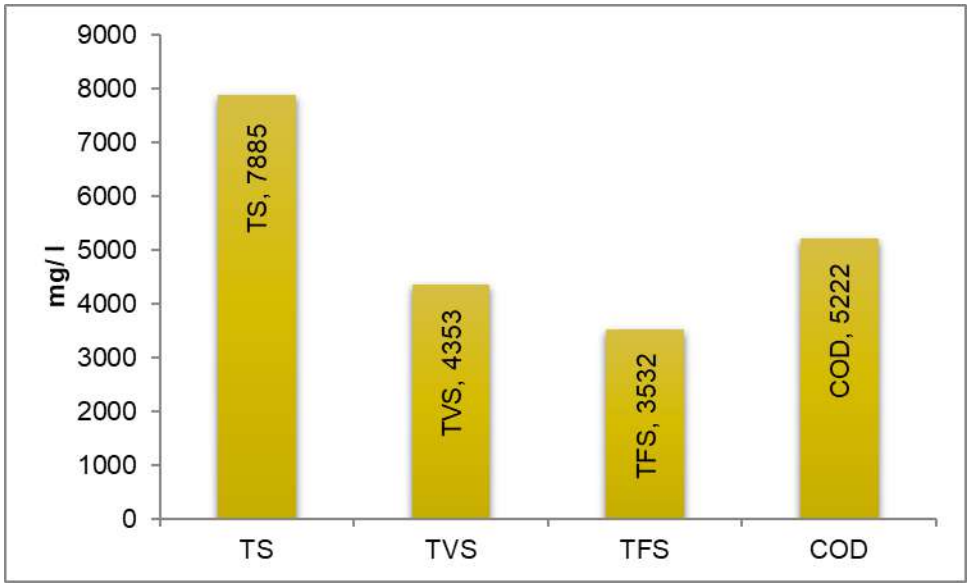

Figure13. Totalsolids(TS)volatilesolids(VS)andTotalFixedSolids(TFS)content in the substrates. Bars represent standard deviations. Digestor outlet stand for Parsley stem waste, floral waste co-digested mixture.

4.3.1. MicrobiologicalAnalysis:

The enumeration of viable heterotrophic bacteria was performed using the pour plate technique, following the guidelines of IS 5402:2012. Digestate samples were serially diluted, plated onto nutrient agar, and incubated at 37 °C for 24–48 hours.

S.No	parameters	units	results
1.	Heterotrophicplate count	CFU/ml	21,82,000

2.	Yeastandmold	CFU/ml	8,95,000

Table6.microbialcount analysis

These comprehensive characterization results provide essential insights into the composition andstability ofthedigestate,informing assessmentsofitspotentialforbeneficial reuse (e.g., as a soil amendment) or the necessity for further treatment prior to disposal.

5.1 OverviewofDigestionPerformance

This study examined the feasibility and efficiency of co-digesting floral waste and parsley stem waste using a 200-liter Deenbandhu biogas digester inoculated with fresh cow dung.Theexperimentaldesignincludedmono-digestiontrials(Bio1:parsleystemwaste;Bio2: floral waste) and a co-digestion trial (Bio3: mixture of both wastes). Biogas production was observed over 21 days, with gas volume estimated using a truncated cone formula.

Among the three setups, co-digestion (Bio3) produced a higher and more consistent biogas yield than mono-digestion. This result confirms the synergistic effect achieved when combining substrates with complementary properties—parsley stems providing structure and fiber,andfloralwasteofferinghighmoistureand carbohydrates.Thesefindingsareconsistent withearlierresearchbyBharathirajaetal.[3]andMata-Alvarezetal.[7],whichdemonstrated increased methane production through co-digestion of balanced substrates.

5.2 PhysicalBehaviorofFeedstocks

A flotation test revealed that parsley stem waste sank immediately due to its fibrous, water-absorbing nature, while floral waste remained buoyant for 7–9 days because of the hydrophobic waxy cuticle. This delayed sinking in floral waste can lead to scum formation, reducedmicrobialcontact,andlowermethaneyield,asalsonotedbySingh&Pradhan[4]and Joshi et al. [5]. In the co-digestion setup, parsley stems helped submerge floral waste by Day 3–4, improving substrate-microbe interaction and slurry homogeneity.

5.3 RoleofCow Dungas Inoculum

Cow dung served as an efficient inoculum, providing a microbial consortium that includes hydrolytic, acidogenic, acetogenic, and methanogenic bacteria. This ensured process stability and rapid initiation of digestion. The buffering capacity of cow dung also helped maintain optimal pH during volatile fatty acid accumulation in early digestion stages, preventing inhibition. These findings align with Verma [15] and Stams&Plugge [16], who emphasized cow dung's effectiveness in small-scale anaerobic systems.

5.4 ChemicalandMicrobialObservations

ChemicalanalysisrevealedthattheCo-digestionmixturehadidealTS,VS,COD,and pH parameters for mesophilic digestion. The balanced C:N ratio and synergistic microbial activity sustained gas production across the 21-day period. Although direct methane measurement was not performed, boiling point tests confirmed the combustibility of the gas, suggesting acceptable methane content in line with Baky&Nazmul [9].

5.5 ProcessStabilityandChallenges

Floatation of floral waste in mono-digestion posed process challenges by delaying microbial hydrolysis. However, co-digestion minimized floatation issues and improved microbialaccess. Thestabletemperatureand useofcow dung buffer supported methanogenic activity throughout the digestion cycle. These challenges are comparable to those outlined by Leitãoetal.[17]andAl-Wahaibietal.[18],whodocumentedsimilarbehaviorsindecentralized AD systems.

5.6 ComparisonwithLiterature

Theresultsofthisstudyalignwiththebroaderliteratureonco-digestion:

Meegoda et al. [1] observed improved yield from substrate synergy.

Singh&Pradhan[4]discussedphysicallimitationsinfloralwastedigestion.

Mata-Alvarez et al. [7] confirmed microbial stabilization and improved buffering in co-digestion systems.

Unlike lab-scale studies using automated measurement (e.g., AMPTS II), this study successfully demonstrated a rural-scale, manual monitoring approach that still yielded productive results.

5.7 PracticalImplications

This system proves that locally available waste can be effectively transformed into biogas using affordable Deenbandhu models. It supports rural electrification, decentralized sanitation, and India's clean energy goals. Digestate analysis also supports its use as a bio-fertilizer,contributingtocircularwasteeconomyprinciples,asrecommendedbyVögelietal. [8]andCurry&Pillay [11].

5.8 LimitationsandFutureResearch

Key limitations:

1. Nomethanepercentageanalysis
2. Nomicrobialcommunityprofiling
3. Notechno-economicassessment

Future studies should integrate methane quantification (Wu et al. [13]), advanced microbialanalysis(Kovácsetal.[12]),andlifecycle/environmentalimpactassessments(Harun et al. [10]) to guide broader implementation and scale-up

7. CONCLUSION

In this study, the anaerobic co-digestion of floral waste and parsley stem waste was investigatedusingcowdungastheinoculuminaDeenbandhu-typebiogasdigester.The

experiment was conducted under mesophilic conditions over a 21-day period, with gas production monitored using a simplified volume displacement method based on the truncated cone formula. Three treatment lines were established: Bio1 (parsley stem mono-digestion), Bio2 (floral waste mono-digestion), and Bio3 (co-digestion of both floral and parsley stem waste). Substrate characterization revealed that the co-digested sample (Bio3) had optimal properties in terms of total solids (TS), volatile solids (VS), and chemical oxygen demand (COD),supportingbettermicrobialactivityanddigestionstability.Theco-digestionslurryalso showed improved physical behavior, with better settling dynamics and fewer issues related to floating materials, which can otherwise hinder microbial access and gas release. Biogas generation data indicated that the co-digestion setup (Bio3) significantly outperformed the mono-digestiontrialsintotalgasyield.WhileBio1andBio2displayedmodestgasproduction foralimitedduration(10 days),Bio3 continuedgenerating biogas consistentlythroughout the 21-dayperiod.Theco-digestionofparsleystems(richinfiberandstructuralcontent)andfloral waste (high in moisture and sugars) created a balanced carbon-to-nitrogen (C/N) ratio that enhanced microbial synergy, leading to greater biogas output. Though the study did not measure methane concentration directly, the combustibility of the gas was verified using boiling point analysis. Observations also confirmed that the co-digested setup maintained a morestablepHrangeandmicrobialpopulation,asevidencedbymesophilicbacterialcountsat the end of the digestion period. Furthermore, physical and biochemical assessments of the digestate post-digestion showed improved organic matter reduction and nutrient recovery in the co-digestion group, supporting its use as a high-quality organic fertilizer. The results validatethehypothesisthatintegratingfloralandparsleystemwastethroughco-digestionwith cowdungenhancesbothbiogasproductionandwastevalorizationefficiency.Thissupportsthe implementationofdecentralizedbiogassystemsinruralandsemi-urbansettingsforsustainable wastemanagementand renewableenergy generation. Overall, thestudy demonstrates thatco-digestionisapracticalandscalableapproachtoimprovingtheenergyyieldandenvironmental benefits of biogas plants using locally available agro-industrial residues.

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