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Enhancing Process Stability Of Fish Waste-Based Anaerobic Digestion Using Various Carbon Additives

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Abstract:
Anaerobic digestion of fish waste can be optimized using carbon additives to enhance biogas production and methane content. This study investigated the effects of coconut shell charcoal, rice husk charcoal, metallic compounds (ferric chloride, manganese chloride, cobalt chloride), and metal-impregnated activated carbon on fish waste digestion. Additionally, apple pomace and grape pomace were evaluated as supplemental substrates. Results showed that 5% (w/v) coconut shell charcoal and rice husk charcoal increased biogas production by 231% and 221%, respectively. Metallic compounds and metal-impregnated activated carbon also enhanced biogas production, with maximum increases of 68%, 159%, and 192% observed with ferric chloride, manganese chloride, and cobalt chloride, respectively. Supplementation with apple pomace and grape pomace at 5% (w/v) increased biogas production by 237.70% and 244.26%, respectively, with a 5% increase in methane content. These findings demonstrate the potential of carbon additives and supplemental substrates to improve the efficiency and stability of fish waste-based anaerobic digestion for enhanced biomethane production.

Background*: India is one among the major global fish producers in the world. Fish is an essential part of our diet, providing essential nutrients. India has a major role in the consumption and export of marine products as it is a peninsular nation with a coastline that stretches for over 7,500 km. According to the Food and Agriculture Organization of the United Nations (FAO), over 178 million tonnes of fish were produced globally in 2021. Fish production in India has increased from 24.42 lakh tonnes in the year 1980-1981 to 175.45 lakh tonnes in the year 2022-2023. Considering the global population growth and the subsequent increase in urbanization and industrialization, the fisheries and aquaculture production has seen a massive increase, driven mainly by the development of fishing technologies. Commercial fishing operations discard an estimated 17.9–39.5 million tons of whole fish each year. In addition to quality losses in the supply chain, ∼130 million tons of fish waste is generated globally each year, representing almost 75 % of total fishery production. Depending on the species, waste produced during fish processing ranges from 20 to 60%. In general, the main types of waste generated by the fish processing industry include head (21.5 ± 4.3 %), liver (5.1 ± 1.9 %), skin (3.3 ± 1.6 %), gut (7.7 ± 3.3 %), fillet/skinned (36.9 ± 8.6 %), backbone (15.3 ± 4.6 %), fins (6.1 ± 2.5 %), roe (4.2 ± 1.7 %), etc. (fish wastes in % of body weight) that can potentially be used as feedstock for biofuels and other value-added products.*

Materials and Methods*: In this prospective randomised controlled study, 60 patients of ASA physical status I and II belonging to age group of 18-60years undergoing elective lower limb surgery under sub-arachnoid block were randomly allocated into 2 groups of 30patients each, Group A (Bupivacaine and Nalbuphine) and Group B (Bupivacaine and Buprenorphine). Group A received 2.8ml of 0.5%(H)Bupivacaine+[0.2 ml (2mg) of Nalbuphine (undiluted) taken in 1ml tuberculin syringe 1mg/0.1ml] and group B received 2.8ml of 0.5%(H)Bupivacaine+0.2ml(60µg) of buprenorphine for spinal anaesthesia. The onset and duration of sensory and motor blockade, 2 segment regression, duration of postoperative analgesia, side-effects and haemodynamic parameters were compared between the groups.* (10)

Results*: The mean time of onset of sensory and motor block, 2 segment regression and duration of motor block was comparable and statistically not significant between the two groups. The duration of postoperative analgesia was significantly prolonged with Buprenorphine compared to Nalbuphine with Bupivacaine ($p<0.05$).* (10)

Conclusion: *Intrathecal Bupivacaine with Buprenorphine 60µg caused prolonged duration of postoperative analgesia when compared to intrathecal Bupivacaine with Nalbuphine 2mg.* (10)

Key Word: *Anaerobic digestion; Fish waste; Carbon additives; Biogas production; Methane content; Process stability*

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I. Introduction

In the modern-day world, energy is quintessential for the economic growth of a country. The level and pattern of utilization of energy from various sources can be considered as an index of the industrial advancement of the country and hence a major determinant of the standard of living of the people. Basically, energy is utilized in three key sectors of our economy, namely agriculture, industry, and for domestic purposes. The raw material for energy production may vary from the most elementary agricultural wastes to highly sophisticated nuclear fuels. The demand for energy increases with a rapid increase in the population (Atelge et al., 2020) (Vardi Venkateswarlu & Chenji Venkatrayulu, 2020). According to estimates, the amount of oil used globally for energy would increase from 14.4 billion tons in 2020 to 26.7 billion tons in 2050. The use of fossil fuels, which have a negative impact on the environment, currently provides more than 84% of the world's energy needs. The sources of energy can broadly be categorized as commercial and non-commercial. Coal, oil, and electricity are the main forms of energy used for commercial production. In the Indian context, commercial sources of energy account for 50% of all energy consumption. 70% of its demand is met by importing oil from the countries. In the 2009- 2010 period, India imported oil worth 86 billion dollars. Dried dung, firewood, and vegetable wastes are some of the commonly employed non-commercial energy sources used in the country. Non-commercial energy sources are renewable; however, commercial energy sources are often exhaustible, with the exception of hydroelectric power. Although the widespread use of commercial energy by humans has improved living standards, it has also brought about a number of issues. Perhaps the most dangerous and crucial of them is the harmful effect it has on the environment. The combustion of fossil fuels has caused serious air pollution problems. Similarly, overuse of firewood causes extensive deforestation and has been linked to a number of respiratory illnesses, particularly in women who spend thousands of years cooking meals all day. Given its clean, green, and infinite nature, biogas from biomass effectively meets the criteria for being a renewable energy source. The term "biomass" describes materials derived from plants and animals that are carbon-based (Ferdeș et al., 2020) 37. The production of renewable energy from agricultural and animal wastes has the potential to offset the usage of fossil fuels, thereby lowering greenhouse gas emissions and averting global warming. Anaerobic digestion has proven to be an efficient method for treating a variety of organic wastes from households, businesses, and farms (Baek et al., 2018) (da Silva et al., 2023) (Chen et al., 2008) (Chen et al., 2022). It is regarded as one of the most affordable ways to produce bioenergy from biomass. Simultaneously, it can address the issue of methane seepage from various industrial and domestic waste materials. The need for renewable sources of energy is particularly important for developing countries which often face energy deficits and rely heavily on fossil fuels, which are not only finite but also contribute significantly to environmental degradation and climate change to meet the energy demand of the expanding population. By diversifying their energy mix with renewable sources such as solar, wind, and hydroelectric power, they can meet the rising energy needs sustainably and reduce their dependence on imported fossil fuels. Additionally, there are numerous socioeconomic advantages to renewable energy. It can create job opportunities, particularly in rural areas where the majority of the population resides (Khayal et al., 2019). Furthermore, boosting energy access and reliability through the construction of renewable energy infrastructure can boost overall quality of life and promote economic growth, especially in isolated and neglected areas. Moreover, renewable energy sources are typically decentralized and modular, allowing for greater energy independence and resilience in developing countries. This aspect is particularly valuable in regions with unreliable or limited access to traditional energy grids. Finally, transitioning to renewable energy aligns with global efforts to mitigate climate change. Developing countries have a crucial role to play in reducing greenhouse gas emissions, and investing in renewable energy can contribute to meeting international climate targets and achieving a sustainable future. Biogas is also known as swamp gas, sewer gas, fuel gas, marsh gas, wet gas (Li et al., 2021). Biogas is a combustible gaseous fuel that is collected from the microbial degradation of organic matter under strict anaerobic condition by a process called as bio methanation or anaerobic digestion (Khayal et al., 2019). It is principally a mixture of methane, the simplest organic compound and carbon dioxide along with other trace gases like hydrogen sulphide(Ao et al., 2024) (Atelge et al., 2020). Methane is the principal gas in biogas burns with clean flame and produces little pollution (Hou et al., 2018). It is also the main component of Natural gas, a fossil fuel. Biogas is one of the renewable sources of energy receiving popularity in rural areas and can be replaced by Natural gas in many applications including cooking, heating, steam production, combined heat and power generation and as a vehicular fuel. Biogas is non-poisonous and non-toxic gas which when mixed with air burns with blue flame without soot at any offensive smell. Statistics rank India to be the third country to have the largest scale of fish cultivation (10.9 million tons as in 2024) next to China and Indonesia (Brooks MS, 2013). Its expansive coastline and diverse water bodies support a thriving marine and freshwater fish industry. With over 1,900 marine and 2,500 freshwater fish species, India's varied geography fosters a rich aquatic biodiversity. Fish and fish products are among the most readily available sources of protein for humans, making up around 80% of all animal protein. There is strong evidence that fish are rich sources of vitamins such as B12 and B6 and minerals, especially iodine and fluorine, which are required for the prevention of goitre and promote strong teeth and bones (Håstein et al., 2006) and (ALKURAIEEF et al., 2022). Countries like India produce over 4 million metric tonnes of seafood waste annually, which is then dumped in landfills, waterways, and other public areas, and its unsafe disposal may pose severe health and environmental hazards (Brooks MS, 2013). The amount of waste generated from the seafood sector begins at the site of harvest itself. In fish waste, more than 75% of the total solids contribute to volatile solids, (Rajendiran et al., 2022) indicating that waste content is organic and suitable for anaerobic digestion. Further, Fish waste showed that the protein content is 4 to 5 times higher than carbohydrate content. The characteristics of Fish waste vary based on the fish varieties and species. Fish Waste contains about 60 to 80% moisture content, and dry matter consists of carbohydrates 5 to 10%, protein 20 to 40%, and lipids 2 to 12% (Ingabire et al., 2023). Fish wastes are high in proteins, amino acids, and minerals; hence, enzymatic treatment is used to extract protein hydrolysate. In fish waste streams, palmitic acid, oleic acid, and monosaturated acids are also prevalent (up to 22%). High moisture content can help fish waste break down and decompose more quickly. Fish waste is biodegradable and breaks down rather quickly in the right conditions because of its high organic content. The decomposition rate can be influenced by factors such as temperature, moisture, oxygen availability, and the presence of microorganisms (Ferdeș et al., 2020) (Simeonov et al., 2021). Solid fish waste consists of head, tails, skin, gut, fins, and frames. Fish waste is used for applications like fertilizer production, amino acids proteins, etc. (Ahuja et al., 2020). The solid fish waste consists of head, tails, skin, gut, fins, and frames (Bücker et al., 2020). These byproducts of the fish processing industry can be a great source of value-added products such as proteins and amino acids, collagen and gelatin, oil, and enzymes (Brooks MS, 2013). These wastes contain proteins (58%), ether extract or fat (19%), and minerals. Also, monosaturated acids, palmitic acid, and oleic acid are abundant in fish waste (22%). This study was aimed at developing a process for improved anaerobic digestion of fish waste utilizing various carbon additives like charcoal from coconut shells, rice husk, and wood ash along with the presence of metal ions. The objective of this work was to develop an improved anaerobic digestion process using fish waste added with carbon additives as the raw material.

II. Experimental

Materials required:

Rotten fish waste, fresh cow dung, tap water, measuring cylinder, petri plates, glass beaker, 1000 mL conical flask, spatula, rubber stopper with one and two holes, rubber tubes, wooden stand for gas collection, grinder, weighing balance, pH meter, biogas slurry, centrifuge tubes, centrifuge, test tubes, Nessler's reagent, distilled water, coconut shell charcoal, rice husk charcoal, wood ash, manganese chloride ($MnCl_2.4H_2O$), cobalt chloride ($CoCl_2.6H_2O$), ferric chloride, activated charcoal, hot air oven, apple pomace, grape pomace, 5% NaOH.

III. Method

Negative control- Cow dung slurry was prepared by mixing 60 g of fresh cow dung with 300 mL of tap water. This mixture was blended together with the aid of a spatula to make a slurry. This slurry was then poured into a 1000-mL conical flask; the final volume of the slurry was then made up to 600 mL with the addition of the required amount of tap water. The initial pH was checked with a pH meter. The 1000-mL conical flask containing the slurry was then closed with a single-holed rubber stopper connected with a tube to an inverted conical flask with water and closed with a double-holed rubber stopper. The outlet tube of the inverted conical flask was placed in a glass beaker containing 100 mL of water to avoid backflow. Fermentation gas produced was collected in the conical by the water displacement method, and the daily displaced volume was measured using a measuring cylinder, which is equal to the daily biogas produced from the particular setup.

Positive control- 30 g of fish waste was taken and mixed with 300 mL of tap water and 60 g of fresh cow dung, which acts as an inoculum. This mixture was blended together with the aid of a spatula to make a slurry. This slurry was then poured into a 1000-mL conical flask; the final volume of the slurry was then made up to 600 mL with the addition of the required amount of tap water. The initial pH was checked with a pH meter. The 1000-mL conical flask containing the slurry was then closed with a single-holed rubber stopper connected with a tube to an inverted conical flask with water and closed with a double-holed rubber stopper. The outlet tube of the inverted conical flask was placed in a glass beaker containing 100 mL of water to avoid backflow. Fermentation gas produced was collected in the conical by the water displacement method, and the daily displaced volume was measured using a measuring cylinder, which is equal to the daily biogas produced from the particular setup.

Determination of biogas production with rotten fish waste as substrate

The test sample was prepared by mixing 30 g of fish waste was taken with 300 mL of tap water and 60 g of fresh cow dung (which acts as an inoculum). This mixture was blended together with the aid of a spatula to make a slurry. This slurry was then poured into a 1000 mL conical flask; the final volume of the slurry was then made up to 600 mL with the addition of the required amount of tap water. The initial pH was checked with a pH meter. The 1000 mL conical flask containing the slurry was then closed with a single-holed rubber stopper connected with a tube to an inverted conical flask with water and closed with a double-holed rubber stopper. The outlet tube of the inverted conical flask was placed in a glass beaker containing 100 mL of water to avoid backflow. Fermentation gas produced was collected in the bottle by the water displacement method, and the daily displaced volume was measured using a measuring cylinder, which is equal to the daily biogas produced from the particular setup.

Detection of ammonia by Nessler's test

After fermentation, 50 mL of slurry was taken and centrifuged at 6000 rpm for 30 minutes. The supernatant was collected and tested with an excessive amount of Nessler's reagent. The colour changed from pale yellow to brown colored precipitate.

Bio-methanation of rotten fish waste with coconut shell charcoal

The test sample was prepared by mixing 30 g of fish waste with 300 mL of tap water, 60 g of fresh cow dung and 1% (w/v) coconut shell charcoal. This mixture was blended together with the aid of a spatula to make a slurry as previously described. This slurry was then poured into a 1000-mL conical flask; the final volume of the slurry was then made up to 600 mL with the addition of the required amount of tap water. The initial pH was checked with pH meter. The 1000-mL conical flask containing the slurry was then closed with a single-holed rubber stopper connected with a tube to an inverted conical flask with water and closed with a double-holed rubber stopper. The outlet tube of the inverted conical flask was placed in a glass beaker containing 100 mL of water to avoid backflow. Fermentation gas produced was collected in the bottle by the water displacement method, and the daily displaced volume was measured using a measuring cylinder, which is equal to the daily biogas produced from the particular setup.

Bio-methanation of rotten fish waste with varying concentration of coconut shell charcoal

The test samples were prepared by mixing 30 g of fish waste with 600 mL of tap water and 60 g of fresh cow dung, which acts as an inoculum, and four individual reaction mixtures prepared with varying concentrations of coconut shell charcoal (1%, 3%, 5%, and 7% w/v). This mixture was blended together with the aid of a spatula to make a slurry. The biogas generated was measured using water displacement method as mentioned in previous sections.

Bio-methanation of rotten fish waste with rice husk charcoal

The test sample was prepared by mixing 30 g of fish waste with 300 mL of tap water, 60 g of fresh cow dung, which acts as an inoculum, and 1% (w/v) rice husk charcoal. This mixture was blended together with the aid of a spatula to make a slurry. This slurry was then poured into a 1000-mL conical flask; the final volume of the slurry was then made up to 600 mL with the addition of the required amount of tap water and the level of biogas generation determined as in previous sections.

Bio-methanation of rotten fish waste with varying concentration of rice husk charcoal

30 g of fish waste was taken and mixed with 300 mL of tap water and 60 g of fresh cow dung, which acts as an inoculum, and four individual reaction mixtures prepared with varying concentrations of rice husk charcoal (1%, 3%, 5%, and 7% w/v) and were used as the test samples to assess the level of biogas production.

Bio-methanation of rotten fish waste with wood ash

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and 1% (w/v) wood ash was used as test sample to assess biogas production.

Bio-methanation of rotten fish waste with varying concentration of wood ash

The test samples were prepared by mixing 30 g of fish waste with 600 mL of tap, water and 60 g of fresh cow dung and two individual reaction mixtures prepared with varying concentrations of wood ash (1%, and 3% w/v). The biogas produced was assessed using water displacement as described in previous sections.

The tests described below were done to investigate the effect of rotten fish waste added with various metal ions in ferric chloride, manganese chloride and cobalt chloride on bio-methanation process. Water displacement method was used throughout the study for determining the level of biogas generation.

Effect of varying concentration of ferric chloride on bio-methanation

The test samples were prepared by mixing 30 g of fish waste with 600 mL of tap water, 60 g of fresh cow dung and four individual reaction mixtures prepared with varying concentrations of iron in ferric chloride (10 ppm, 25 ppm, 50 ppm, and 70 ppm).

Effect of varying concentration of manganese chloride on bio-methanation

The slurry prepared by mixing 30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and five individual reaction mixtures prepared with varying concentrations of manganese in manganese chloride (10 ppm, 25 ppm, 50 ppm, 70 ppm, and 90 ppm) were used as test samples for determining impact on biogas production.

Effect of varying concentration of cobalt chloride on bio-methanation

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and five individual reaction mixtures prepared with varying concentrations of cobalt in cobalt chloride (10 ppm, 25 ppm, 50 ppm and 70 ppm) were used as test samples.

Synergistic effect of metal ions (in ferric chloride, manganese chloride, cobalt chloride) on bio-methanation of rotten fish waste

30 g of fish waste was taken and mixed with 300 mL of tap water, 60 g of fresh cow dung and two individual reaction mixtures prepared with varying concentrations of ferric chloride, manganese chloride, and cobalt chloride to explore the synergistic effect of these metal ions. This mixture was blended together with the aid of a spatula to make a slurry. This slurry was then poured into a 1000-mL conical flask; the final volume of the slurry was then made up to 600 mL with the addition of the required amount of tap water. The initial pH was checked with a pH meter. The 1000 mL conical flask containing the slurry was then closed with a single-holed rubber stopper connected with a tube to an inverted conical flask with water and closed with a double-holed rubber stopper. The outlet tube of the inverted conical flask was placed in a glass beaker containing 100 mL of water to avoid backflow. Fermentation gas produced was collected in the bottle by the water displacement method, and the daily displaced volume was measured using a measuring cylinder, which is equal to the daily biogas produced from the particular setup.

The following section investigates the effect of metal ions (in ferric chloride, manganese chloride and cobalt chloride) impregnated activated charcoal on the bio-methanation of rotten fish waste. To prepare metal ion impregnated charcoal, 5 g of respective metal chloride and 5 g charcoal were added to 100mL water, mixed and kept for an hour. The mixture was then centrifuged and the residue obtained was dried in a hot air oven and kept it in dry glass bottles until further use.

Effect of varying concentration of ferric chloride impregnated activated charcoal on bio-methanation

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and four individual reaction mixtures prepared with varying concentrations of iron in ferric chloride (10 ppm, 25 ppm, 50 ppm, and 70 ppm) were used as control for the study. 30g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and four individual reaction mixtures prepared with varying concentrations of ferric chloride impregnated activated charcoal (10 ppm, 25 ppm, 50 ppm, and 70 ppm) were used as test samples.

Effect of varying concentration of manganese chloride impregnated activated charcoal on bio-methanation

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and four individual reaction mixtures prepared with varying concentrations of manganese in manganese chloride (10 ppm, 25 ppm, 50 ppm, 70 ppm and 90 ppm) were used as control for the study. 30g of fish waste mixed with 300 mL of tap water, 60 g of fresh cow dung and four individual reaction mixtures prepared with varying concentrations of manganese chloride impregnated activated charcoal (10 ppm, 25 ppm, 50 ppm, 70 ppm and 90 ppm) were used as test samples.

Effect of varying concentration of cobalt chloride impregnated activated charcoal on bio-methanation

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and four individual reaction mixtures prepared with varying concentrations of cobalt in cobalt chloride (10 ppm, 25 ppm, 50 ppm, and 70 ppm) were used as control for the study. 30g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and four individual reaction mixtures prepared with varying concentrations of cobalt chloride impregnated activated charcoal (10 ppm, 25 ppm, 50 ppm, and 70 ppm) were used as test samples.

Synergistic effect of metal ions (in ferric chloride, manganese chloride and cobalt chloride) impregnated activated charcoal on bio-methanation of rotten fish waste

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and two individual reaction mixtures prepared with varying concentrations of ions in ferric chloride, manganese chloride and cobalt chloride were used as control to find out the synergistic effect of these metal ions. While, 30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and two individual reaction mixtures prepared with varying concentrations of ferric chloride, manganese chloride, and cobalt chloride impregnated activated charcoal were employed as the test sample for the study.

Supplementation of apple pomace (carbon-nitrogen optimization)

30 g of fish waste mixed with 300 mL of tap water, 60 g of fresh cow dung and 1% (w/v) apple pomace was used as test sample to check for biogas production as against positive (30 g of fish waste mixed with 600 mL of tap water and 60 g of fresh cow dung) and negative (cow dung slurry prepared by mixing 60 g of fresh cow dung with 600 mL of tap water) control groups.

Bio-methanation of rotten fish waste with varying concentration apple pomace

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and five individual reaction mixtures prepared with varying concentrations of apple pomace: 1%, 2%, 3%, 5%, and 7% (w/v) were used as test samples for the study.

Supplementation of grape pomace (carbon-nitrogen optimization)

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and 1% (w/v) grape pomace was used as test sample for the study.

Bio-methanation of rotten fish waste with varying concentration grape pomace

30 g of fish waste mixed with 600 mL of tap water, 60 g of fresh cow dung and four individual reaction mixtures prepared with varying concentrations of grape pomace [1%, 3%, 5%, and 7% (w/v)] were used as test samples.

The following section describes the method adopted for the determination of methane content in biogas using 5% NaOH scrubbing method.

Determination of methane content in biogas with rotten fish waste as substrate

30 g of fish waste was mixed with 300 mL of tap water and 60 g of fresh cow dung. This mixture was blended together with the aid of a spatula to make a slurry. This slurry was then poured into a 1000 mL conical flask and the final volume of the slurry was made up to 600 mL by adding required amount of tap water. The initial pH was checked with a pH meter. An inverted glass bottle was filled with water containing 5% NaOH, and the quantity of methane gas was measured by the water displacement method.

Determination of methane content in biogas by the effect of coconut shell charcoal

30 g of fish waste was mixed with 600 mL of tap water, 60 g of fresh cow dung and 5% (w/v) coconut shell charcoal. This mixture was blended together with the aid of a spatula to make a slurry and methane content determined as described in the previous section.

Determination of methane content in biogas by the effect of apple pomace

30 g of fish waste was mixed with 300 mL of tap water, 60 g of fresh cow dung and 5% (w/v) apple pomace. This mixture was blended together with the aid of a spatula to make a slurry, made up to final volume of 600 mL and methane content determined as described in the section 3.21.

IV. Results And Discussion

Determination of biogas production with rotten fish waste as substrate

Figure 4.1. Determination of biogas production with rotten fish waste as substrate

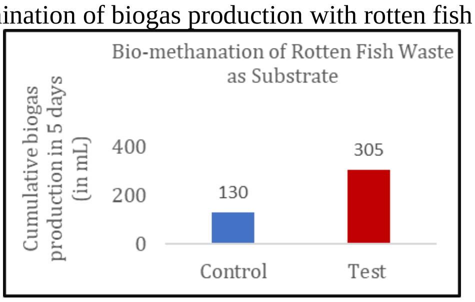

Table 4.1. Determination of biogas production with Rotten fish waste as substrate

Sample	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control (%)
Control	60g fresh cow dung made up to 600mL with tap water	130	
Test	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	134.61

Figure 4.1 shows bio-methanation of fish waste as a substrate. It was found that there was cumulative biogas production 305 mL from 30g fish waste and 60 g cow dung with 98 functional hours. The fermentation stops after 119 hours due to over production of ammonia and buildup of excess alkalinity.

An optimal ammonia concentration improves the process stability by improving the buffering capacity of the methanogenic medium. It is worth noting that ammonia is formed as a by-product of biological degradation of nitrogenous portion of substrates, such as proteins and urea (Kayhanian, 1999). Ammonia is an important nutritional requirement for the bacteria to carry out various metabolic processes. Nonetheless, excessive ammonia concentration is found to be an inhibitor to anaerobic digestion and microbial activity within digester.

Detection of ammonia by Nessler's test

Figure 4.2. Detection of ammonia by Nessler's test showing presence of ammonia detected by changed from pale yellow to brown coloured precipitate

Fig 4.2 shows detection of ammonia by Nessler's test. As per the test result the colour changed from pale yellow to brown coloured precipitate. This indicated the presence of ammonia in the fermentation mixture.

Carbon additives can play a significant role in enhancing biogas production by improving the digestion process. These additives, such as carbon-rich materials like biochar, can provide a surface for microbes to attach and thrive, increasing microbial activity and aiding in the breakdown of organic matter. Additionally, carbon additives can also help balance the C/N ratio in the digestion process, promoting a healthier microbial community and preventing the accumulation of ammonia.

Carbon additives, can further support and enhance DIET in biogas production systems. These additives provide a conductive surface for microbial attachment and growth, promoting direct electron transfer between microorganisms. Carbon additives can also help to adsorb inhibitory compounds and create a favorable microenvironment for the microbial community involved in the DIET.

The anaerobic methanogenesis process is mediated by microorganisms of the three major bacterial groups, which form a symbiotic relationship between microorganisms, thus overcoming the thermodynamic barriers of the metabolic process. In symbiotic relationships, interspecies electron transfer (IET) is a new type of mutualistic symbiosis that has been discovered in recent years. Electron donor microorganisms transfer electrons to electron acceptor microorganisms by direct means of cell contact or indirect pathways mediated by intermediates, thus enabling metabolic processes that are difficult for a single microorganism to accomplish. IET can be divided into Direct Interspecies Electron Transfer (DIET) and Indirect Interspecies Electron Transfer (IIET) according to the different modes of electron transfer.

Coconut shell charcoal is a carbon-rich material that can be used as an extra substrate for growth of anaerobic bacteria. Coconut shells are available and as the coconut board. The latest commodity prize of coconut shell charcoal is rupees 36500 per ton.

Bio-methanation of rotten fish waste with coconut shell charcoal

Figure 4.3. Bio-methanation of rotten fish waste with coconut shell charcoal

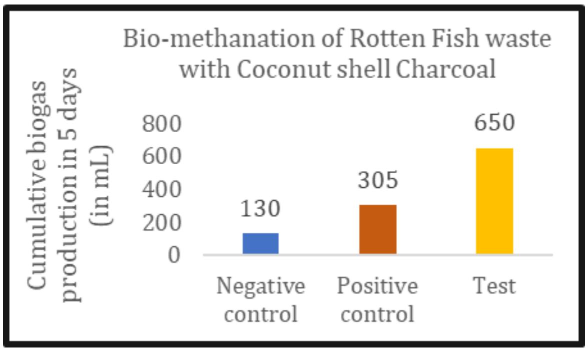

Table 4.2. Determination of biogas production by the addition of coconut shell charcoal

Sample	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control (%)
Negative control	60g fresh cow dung made up to 600mL with tap water	130	
Positive control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test	30g fish waste +60g fresh cow dung + 1%(w/v) Coconut shell charcoal, made up to 600mL with tap water	650	113.114%

Figure 4.3 shows the data regarding bio methanation of fish waste in the presence of 1%(w/v) Coconut shell charcoal. Cumulative biogas production was 650mL in 100 and hours 30minutes and fermentation stopped after 116 hours 30 minutes. Percentage increase with respect to positive control was 113.11%. Also, optimization was done using 1%, 3%, 5% and 7% coconut shell charcoal levels. It is clear from Fig.4.4 that the addition of 5% coconut shell charcoal produced more biogas compared to other concentrations (table 4.3). It was found that the cumulative biogas production was 1010mL.

Bio-methanation of rotten fish waste with varying concentrations of coconut shell charcoal

Figure 4.4. Bio-methanation of rotten fish waste with varying concentrations of coconut shell charcoal

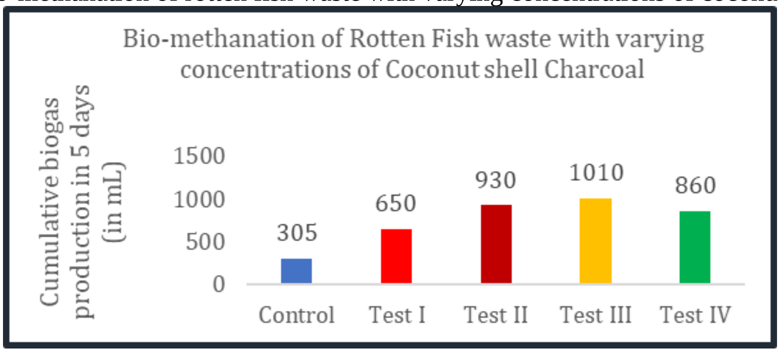

Table 4.3 Bio-methanation of rotten fish waste with varying concentrations of coconut shell charcoal

Sample	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control (%)
Control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste +60g fresh cow dung + 1%(w/v) Coconut shell charcoal, made up to 600mL with tap water	650	113.11%
Test II	30g fish waste + 60g fresh cow dung + 3%(w/v) Coconut	930	204.91%

	shell charcoal, made up to 600mL with tap water		
Test III	30g fish waste + 60g fresh cow dung + 5%(w/v) Coconut shell charcoal, made up to 600mL with tap water	1010	231.14%
Test IV	30g fish waste + 60g fresh cow dung + 7%(w/v) Coconut shell charcoal, made up to 600mL with tap water	860	181.96%

Bio-methanation of rotten fish waste with rice husk charcoal

Figure 4.5. Bio-methanation of rotten fish waste with rice husk charcoal

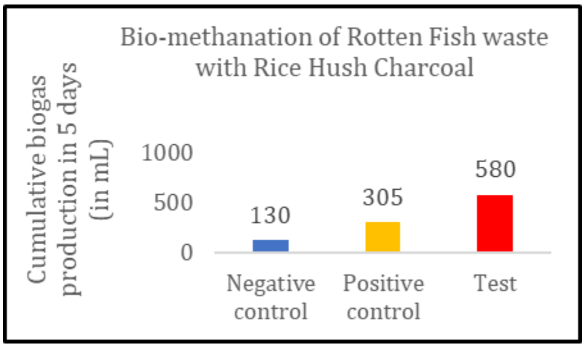

Table 4.4 Determination of Biogas production by the addition of rice husk charcoal

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to positive control
Negative control	60g fresh cow dung made up to 600mL with tap water	130	
Positive control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test	30g fish waste + 60g fresh cow dung + 1%(w/v) Rice Husk charcoal, made up to 600mL with tap water	580	90.16%

The lignocellulosic structure can act as a complicated carbon source, promoting the growth of a wide variety of microorganisms within the digester. The outer coating of rice grains is used to make rice husk charcoal. It is high in cellulose and hemicellulose, which anaerobic microorganisms may easily breakdown. Rice husk charcoal can supply readily available carbon molecules, encouraging microbial activity and biogas production. The annual production of rice husk in India is around 31.40 million tones. The main producers of the rice husk in India are Punjab, Haryana and UP. This rice husk converted as rice husk charcoal and used as carbon additives in anaerobic digestion.

Also, the effect of rice husk charcoal on biogas production using rotten fish waste was determined and there is significant increase in the production compared to control (90.16%) as shown in figure 4.5 and table 4.4.

Bio-methanation of rotten fish waste with varying concentrations of rice husk charcoal

Figure 4.6. Bio-methanation of rotten fish waste with varying concentrations of rice husk charcoal

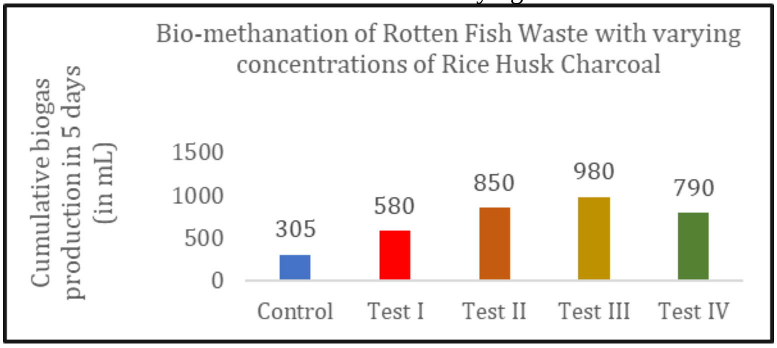

Table 4.5 Bio-methanation of rotten fish waste with varying concentrations of rice husk charcoal

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste + 60g fresh cow dung + 1%(w/v) Rice Husk charcoal, made up to 600mL with tap water	580	90.16%
Test II	30g fish waste + 60g fresh cow dung + 3%(w/v) Rice Husk charcoal, made up to 600mL with tap water	850	178.68%
Test III	30g fish waste + 60g fresh cow dung + 5%(w/v) Rice Husk charcoal, made up to 600mL with tap water	980	221.31%
Test IV	30g fish waste+ 60g fresh cow dung + 7%(w/v) Rice Husk charcoal, made up to 600mL with tap water	790	159%

Fig.4.6 shows the data of cumulative biogas production after the addition of rice husk charcoal in varying concentrations. It was evident from the figure that 5% rice husk charcoal was the optimum concentration for bio-methanation using rotten fish waste as a substrate (980mL). There was 221.31% increase in the production of biogas when compared to control (table 4.5).

Many operating parameters, including material content, temperature, pH, C/N ratio, retention period, etc., affect the gas yield in the anaerobic digestion process. Numerous studies looked into how operating conditions affected the production of biogas and published their findings. Using food waste as feed, Kim et al. (2006) looked into the effects of temperature and hydraulic retention time on anaerobic digestion. They found that, at 50°C and over a 12-day hydraulic retention period, anaerobic digestion performance and food waste digestion efficiency improved. The impact of pH on the production of biogas from spoilt milk was investigated by Sivakumar et al. (2012). Studies using substrates with varying pH values (5-8) revealed that the substrate with a pH of 7 produced a higher yield of biogas.

Bio-methanation of rotten fish waste with wood ash

Figure 4.7. Bio-methanation of rotten fish waste with wood ash

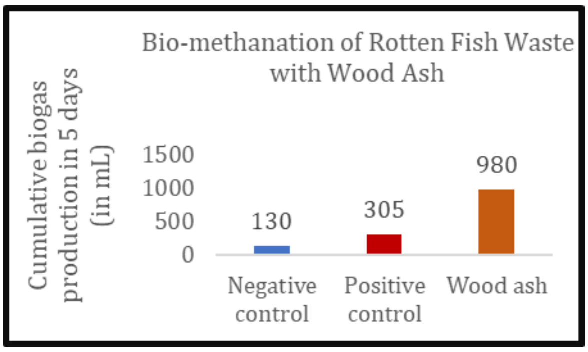

Table 4.6 Determination of Biogas production by the addition of wood ash

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to positive control
Negative control	60g fresh cow dung made up to 600mL with tap water	130	
Positive control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test	30g fish waste + 60g fresh cow dung + 1%(w/v) wood ash, made up to 600mL with tap water	980	221.311%

Fig.4.7 shows the effect of wood ash on biogas production. Since the wood ash primarily consists of calcium and other alkaline elements, this material can be used to increase the pH of the anaerobic digestate and promote the volatilization of NH_3. It is clear from the figure that there is an increase in the production of biogas after the addition of wood ash (221.311%). It is evident from Table 4.6 that, the fermentation mixture containing 1% wood ash produced more biogas when compared to other concentrations. The cumulative biogas production was found to be 980mL and there was 221.311% increase in the production of biogas when compared to the control.

The term heavy metals refer to metals and metalloids having densities greater than 5 g cm^{-3} and is usually associated with pollution and toxicity although some of these elements (essential metals) are required by micro-organisms at low concentrations. Heavy metals toxicity and the danger of their bioaccumulation in the food chain represent one of the major environmental and health problems of our modern society. Heavy metals act as cofactors for key enzymes that catalyze biochemical reactions during anaerobic digestion processes of organic matter. Heavy metals like copper, nickel, zinc, cadmium, chromium and lead have been found to be inhibitory and under certain conditions toxic in biochemical reactions depending on their concentrations. Heavy metals like iron may also exhibit stimulatory effects, but these effects are not studied in detail.

Bio-methanation of rotten fish waste with varying concentrations of wood ash

Figure 4.8. Bio-methanation of rotten fish waste with varying concentrations of wood ash

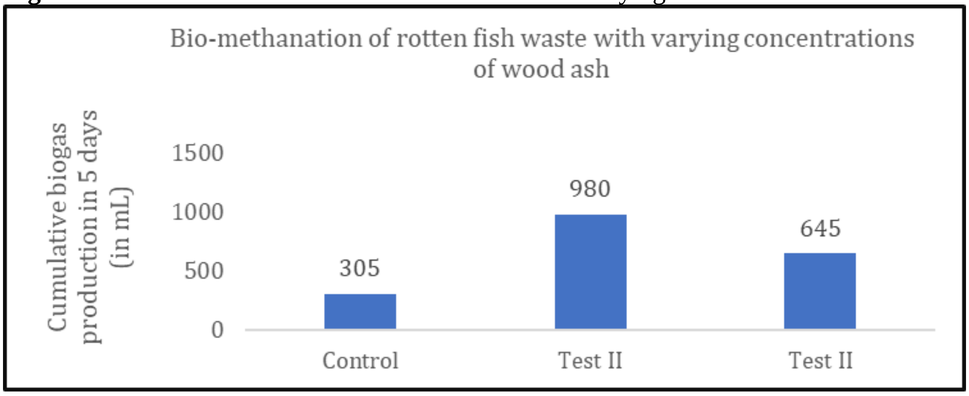

Table 4.7 Bio-methanation of rotten fish waste with varying concentrations of wood ash

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste + 60g fresh cow dung + 1%(w/v) wood ash, made up to 600mL with tap water	920	201.63%
Test II	30g fish waste + 60g fresh cow dung + 3%(w/v) wood ash, made up to 600mL with tap water	645	111.47%

As can be seen from table 4.7, there was 201.63 % increase in biogas production on supplementation with 1% w/v of wood ash compared to control.

Effect of varying concentrations of ferric chloride on bio-methanation

Figure 4.9. Effect of varying concentrations of ferric chloride on bio-methanation

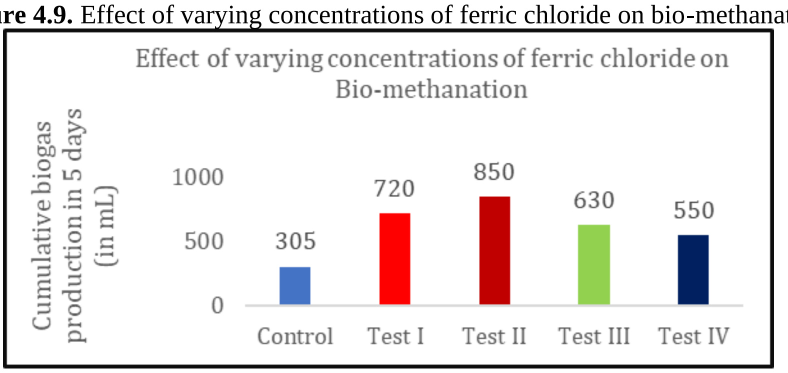

Table 4.8 Effect of varying concentrations of ferric chloride on bio-methanation

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste + 60g fresh cow dung + 0.0174 g ferric chloride (10ppm iron), made up to 600mL with tap water	720	136.06%
Test II	30g fish waste + 60g fresh cow dung + 0.0436 g ferric chloride (25ppm iron), made up to 600mL with tap water	850	178.68%

Test III	30g fish waste + 60g fresh cow dung + 0.0872 g ferric chloride (50ppm iron), made up to 600mL with tap water	630	106.55%
Test IV	30g fish waste+ 60g fresh cow dung + 0.122 g ferric chloride (70ppm iron), made up to 600mL with tap water	550	80.32%

Fig 4.9 shows the effect of Ferric chloride on bio-methanogenic pathway and it was found that there is 178.68% (table 4.8) increase in biogas production when 25ppm ferric ion (0.0436g ferric chloride) is used. Also, it is evident from Fig 4.10 that the addition of Manganese chloride produces 159% more biogas when compared to the control. Also, it is evident from Fig 4.11 and table 4.10 that the addition of Cobalt chloride produced 192% more biogas when compared to the control.

Effect of varying concentrations of manganese chloride on bio-methanation

Figure 4.10. Effect of varying concentrations of manganese chloride on bio-methanation

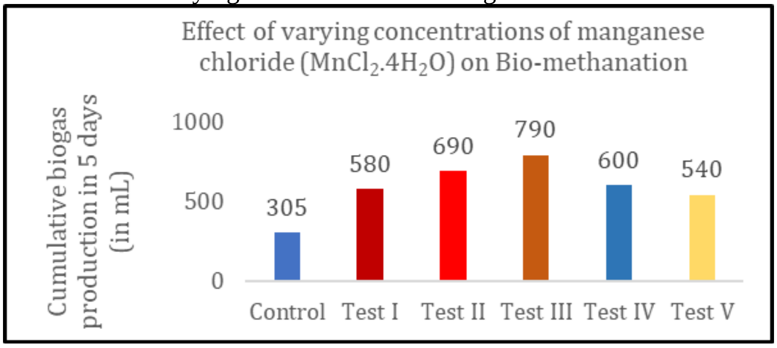

Table 4.9 Effect of varying concentrations of manganese chloride on Bio-methanation

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste + 60g fresh cow dung + 0.0216 g manganese chloride (10ppm manganese), made up to 600mL with tap water	580	90.16%
Test II	30g fish waste + 60g fresh cow dung + 0.054 g manganese chloride (25ppm manganese), made up to 600mL with tap water	690	126.22%
Test III	30g fish waste + 60g fresh cow dung + 0.108 g manganese chloride (50ppm manganese), made up to 600mL with tap water	790	159%
Test IV	30g fish waste+ 60g fresh cow dung + 0.151 g manganese chloride (70ppm manganese), made up to 600mL with tap water	600	96.72%
Test V	30g fish waste+ 60g fresh cow dung + 0.195 g manganese chloride (90ppm manganese), made up to 600mL with tap water	540	77%

Effect of varying concentrations of cobalt chloride on bio-methanation

Figure 4.11. Effect of varying concentrations of cobalt chloride on bio-methanation

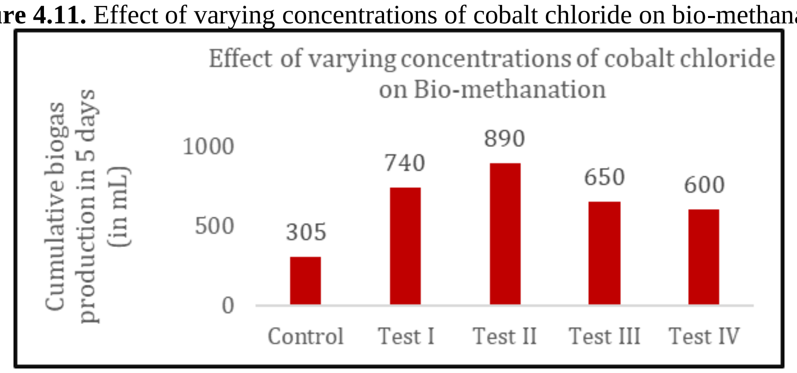

Table 4.10. Effect of varying concentrations of cobalt chloride on Bio-methanation Fermentation mixture containing metal ion at 25ppm levels resulted in highest yield of biogas (table 4.10).

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste + 60g fresh cow dung + 0.024g cobalt chloride (10ppm cobalt), made up to 600mL with tap water	740	142.62%
Test II	30g fish waste + 60g fresh cow dung + 0.061g cobalt chloride (25ppm cobalt), made up to 600mL with tap water	890	192%
Test III	30g fish waste + 60g fresh cow dung + 0.121g cobalt chloride (50ppm cobalt), made up to 600mL with tap water	650	113.11%
Test IV	30g fish waste+ 60g fresh cow dung + 0.169g cobalt chloride (70ppm cobalt), made up to 600mL with tap water	600	96.72%

Synergistic effect of metal ions (Ferric chloride, manganese chloride, cobalt chloride) on bio-methanation of rotten fish waste

Figure 4.12. Synergistic effect of metal ions on bio-methanation of rotten fish waste

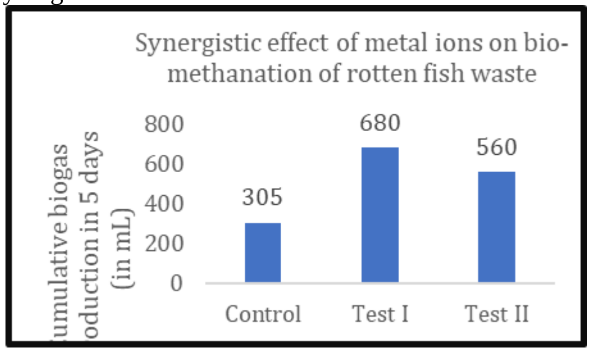

Table 4.11. Synergistic effect of metal ions (Ferric chloride, manganese chloride & cobalt chloride) on bio-methanation of rotten fish waste

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste + 60g fresh cow dung + 0.043g ferric chloride (25ppm iron), 0.061g cobalt chloride (25ppm cobalt), 0.108g manganese chloride (50ppm manganese), made up to 600mL with tap water	680	122.95%
Test II	30g fish waste + 60g fresh cow dung + 0.087g ferric chloride (50ppm), 0.121g cobalt chloride (50ppm cobalt), 0.151g manganese chloride (70ppm manganese), made up to 600mL with tap water	560	83.60%

Synergistic effect of ferric chloride, manganese chloride and cobalt chloride were studied (Fig 4.12) and it was clear that the addition of 25ppm ferric, 50ppm manganese along with 25ppm cobalt produced maximum biogas (680mL) (table 4.11).

Effect of varying concentrations of ferric chloride impregnated activated charcoal on bio-methanation

Figure 4.13. Effect of varying concentrations of ferric chloride impregnated activated charcoal on bio-methanation

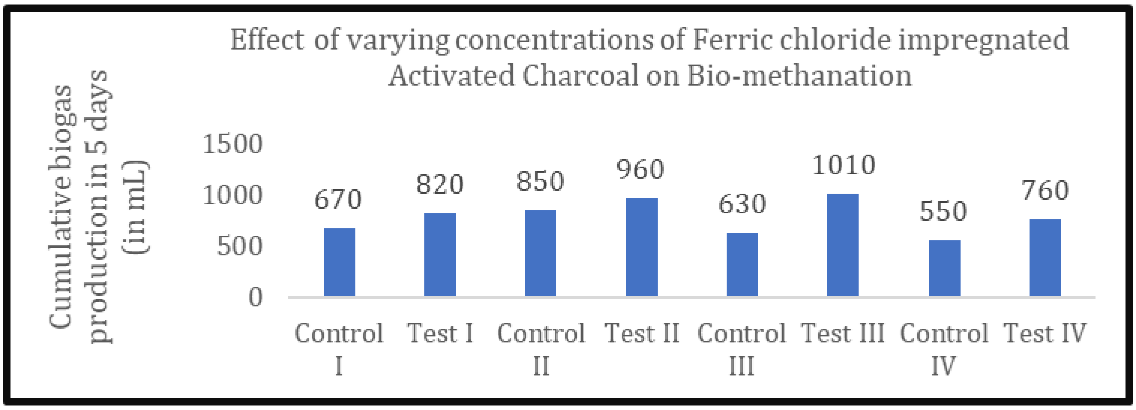

Table 4.12. Effect of varying concentrations of ferric chloride impregnated activated charcoal on bio-methanation

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control I	30g fish waste +60g fresh cow dung+ 0.0174g ferric chloride (10ppm iron), made up to 600mL tap water	670	
Test I	30g fish waste + 60g fresh cow dung + ferric chloride (10ppm iron) impregnated activated charcoal (0.0174g ferric chloride), made up to 600mL with tap water	820	22.38%
Control II	30g fish waste +60g fresh cow dung+ 0.043g ferric chloride (25ppm iron), made up to 600mL tap water	850	
Test II	30g fish waste + 60g fresh cow dung + ferric chloride (25ppm iron) impregnated activated charcoal (0.043g ferric chloride), made up to 600mL with tap water	960	12.94%
Control III	30g fish waste +60g fresh cow dung+ 0.087g ferric chloride (50ppm iron), made up to 600mL tap water	630	
Test III	30g fish waste + 60g fresh cow dung + ferric chloride (50ppm iron) impregnated activated charcoal (0.087g ferric chloride), made up to 600mL with tap water	1010	60.31%
Control IV	30g fish waste+60g fresh cow dung + 0.122g ferric chloride (70ppm iron), made up to 600 mL tap water	550	
Test IV	30g fish waste+ 60g fresh cow dung +ferric chloride (70ppm iron) impregnated activated charcoal (0.122g ferric chloride), made up to 600mL with tap water	760	38.18%

Metal impregnated charcoal was found to be excellent for maximizing biogas production. It is evident that 50ppm ferric impregnated carbon produced more biogas compared to the control (60.31% increase) (table 4.12). And by the addition of 70ppm manganese impregnated carbon, there was 58.33% increase (table 4.13) in biogas production. Also, it is evident from Fig 4.15 that the addition of cobalt chloride produced 56.92% (table 4.14) more biogas when compared to the control.

Effect of varying concentrations of manganese chloride impregnated activated charcoal on bio-methanation

Figure 4.14. Effect of varying concentrations of manganese chloride impregnated activated charcoal on bio-methanation

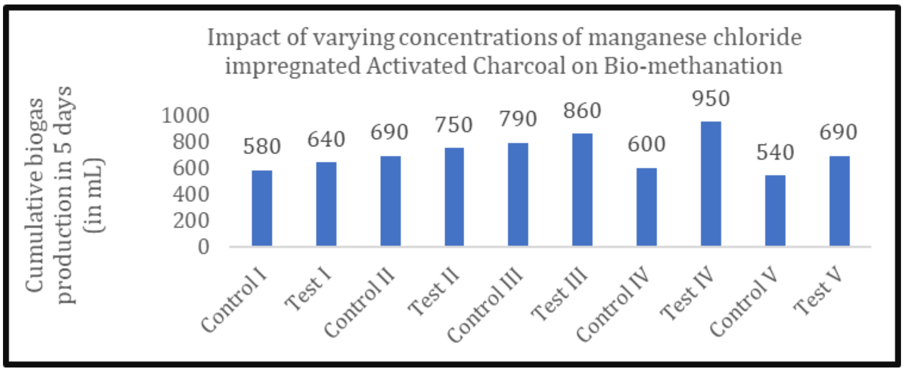

Table 4.13. Effect of varying concentrations of manganese chloride impregnated Activated Charcoal on Bio-methanation

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control I	30g fish waste +60g fresh cow dung+ 0.021g manganese chloride (10ppm manganese ion), made up to 600mL tap water	580	
Test I	30g fish waste + 60g fresh cow dung+ manganese chloride (10ppm manganese) impregnated activated charcoal (0.021g manganese chloride), made up to 600mL with tap water	640	10.34%
Control II	30g fish waste +60g fresh cow dung+ 0.054g manganese chloride (25ppm manganese), made up to 600mL tap water	690	
Test II	30g fish waste + 60g fresh cow dung + manganese chloride (25ppm manganese) impregnated activated charcoal (0.054g manganese chloride), made up to 600mL with tap water	750	8.69%
Control III	30g fish waste +60g fresh cow dung+ 0.108g manganese chloride (50ppm manganese), made up to 600mL tap water	790	
Test III	30g fish waste + 60g fresh cow dung + manganese chloride (50ppm manganese) impregnated activated charcoal (0.108g manganese chloride), made up to 600mL with tap water	860	8.86%
Control IV	30g fish waste+60g fresh cow dung+ 0.151g manganese chloride (70ppm manganese), made up to 600 mL tap water	600	
Test IV	30g fish waste+ 60g fresh cow dung + manganese chloride (70ppm manganese) impregnated activated charcoal (0.151g manganese chloride), made up to 600mL with tap water	950	58.33%
Control V	30g fish waste+60g fresh cow dung+ 0.194g manganese chloride (90ppm manganese), made up to 600 mL tap water	540	
Test V	30g fish waste+ 60g fresh cow dung + manganese chloride (90ppm manganese) impregnated activated charcoal (0.194g manganese chloride), made up to 600mL with tap water	690	27.77%

Effect of varying concentrations of cobalt chloride impregnated activated charcoal on bio-methanation

Figure 4.15. Effect of varying concentrations of cobalt chloride impregnated activated charcoal on bio-methanation

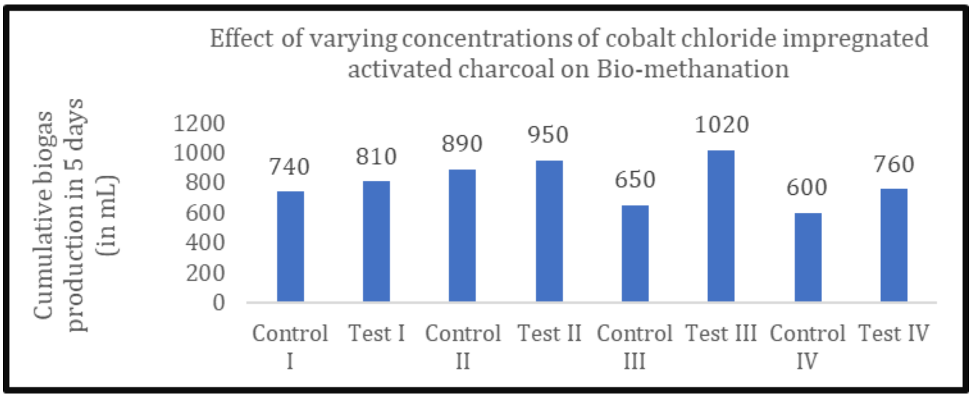

Table 4.14. Effect of varying concentrations of cobalt chloride impregnated activated charcoal on Bio-methanation

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	30g fish waste + 60g fresh cow dung + 0.024g cobalt chloride (10ppm cobalt), made up to 600mL with tap water	740	
Test I	30g fish waste + 60g fresh cow dung + cobalt chloride (10ppm cobalt) impregnated activated charcoal (0.024g cobalt chloride), made up to 600mL with tap water	810	9.45%
Control II	30g fish waste + 60g fresh cow dung + 0.06g cobalt chloride (25ppm cobalt), made up to 600mL with tap water	890	
Test II	30g fish waste + 60g fresh cow dung + cobalt chloride (25ppm cobalt) impregnated activated charcoal (0.06g cobalt chloride), made up to 600mL with tap water	950	6.74%
Control III	30g fish waste + 60g fresh cow dung + 0.121g cobalt chloride (50ppm cobalt), made up to 600mL with tap water	650	
Test III	30g fish waste + 60g fresh cow dung + cobalt chloride (50ppm cobalt) impregnated activated charcoal (0.121g cobalt chloride), made up to 600mL with tap water	1020	56.92%
Control IV	30g fish waste+ 60g fresh cow dung + 0.169g cobalt chloride (70ppm cobalt), made up to 600mL with tap water	600	
Test IV	30g fish waste+ 60g fresh cow dung + cobalt chloride (70ppm cobalt) impregnated activated charcoal (0.169g cobalt chloride), made up to 600mL with tap water	760	26.66

Synergistic effect of metal ions (ferric chloride, manganese chloride and cobalt chloride) impregnated activated charcoal on bio-methanation of rotten fish waste

Figure 4.16. Synergistic effect of metal ions (ferric chloride, manganese chloride and cobalt chloride) impregnated activated charcoal on bio-methanation of rotten fish waste

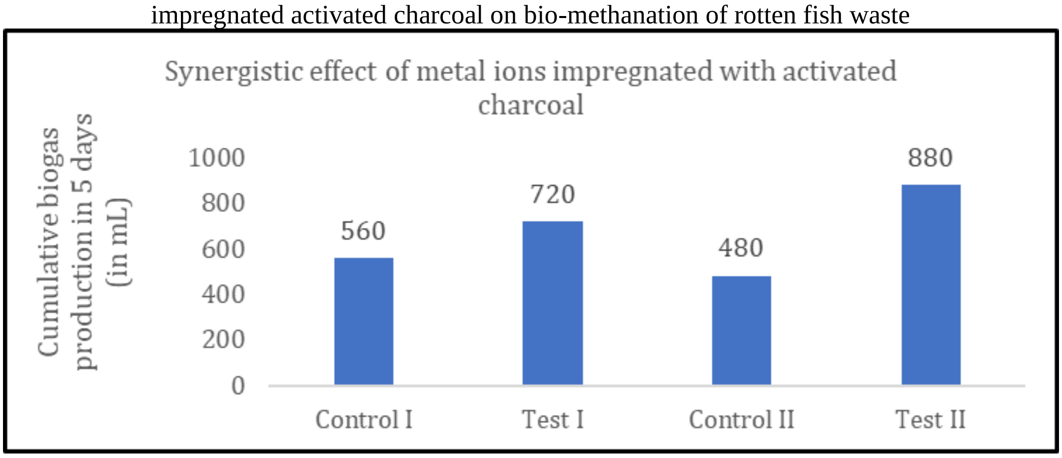

Table 4.15. Synergistic effect of metal ions impregnated activated charcoal

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control I	30g fish waste+60g fresh cow dung+ 0.087g ferric chloride (50ppm iron), 0.121g cobalt chloride (50ppm cobalt), 0.151g manganese chloride (70ppm manganese), made up to 600mL with tap water	560	
Test I	30g fish waste+60g fresh cow dung+ 0.087g ferric chloride (50ppm iron), 0.121g cobalt chloride (50ppm cobalt), 0.151g manganese chloride (70ppm manganese) impregnated activated charcoal, made up to 600mL with tap water	720	28.57%
Control II	30g fish waste + 60g fresh cow dung + 0.122g ferric chloride (70ppm), 0.169g cobalt chloride (70ppm cobalt), 0.194g manganese chloride (90ppm manganese) impregnated activated charcoal, made up to 600mL with tap water	480	
Test II	30g fish waste + 60g fresh cow dung + 0.122g ferric chloride (70ppm iron), 0.169g cobalt chloride (70ppm cobalt), 0.194g manganese chloride (90ppm manganese) impregnated activated charcoal, made up to 600mL with tap water	880	83.33%

Synergistic effect of metal ion impregnated charcoal was determined (Fig 4.16 and table 4.15) and it was found that 70ppm Ferric impregnated carbon, 90ppm Manganese impregnated carbon along with 70ppm carbon impregnated Cobalt had maximum production of biogas (880mL).

Supplementation of apple pomace (carbon-nitrogen optimization)

Figure 4.17. Supplementation of apple pomace (carbon-nitrogen optimization)

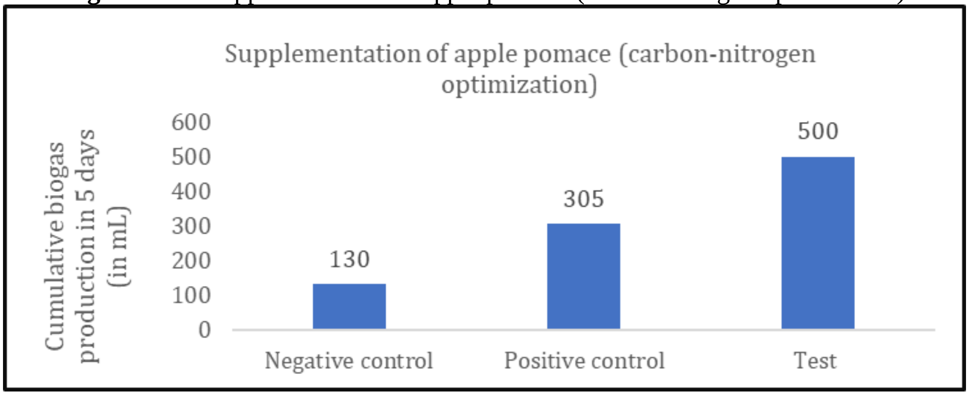

Table 4.16. Supplementation of apple pomace

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to positive control
Negative control	60g fresh cow dung made up to 600mL with tap water	130	
Positive control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test	30g fish waste +60g fresh cow dung + 1%(w/v) apple pomace, made up to 600mL with tap water	500	63.93%

An optimum carbon to nitrogen (C/N) ratio is crucial for effective digestion of substrate and biogas production. The ideal carbon to nitrogen (C: N) ratio for anaerobic digestion ranges from approximately 20:1 to 30:1. The addition of co-digestion materials with higher carbon (or nitrogen) contents than manure feedstock can improve the C:N ratio, thereby increasing methane production. Extremely high or low C:N ratio could inhibit methanogenic population and hence methane generation rate, which would favour accumulation of volatile fatty acids (VFAs) and ammonia in digestion solution.

Bio-methanation of rotten fish waste with varying concentrations of apple pomace

Figure 4.18. Bio-methanation of rotten fish waste with varying concentrations of apple pomace

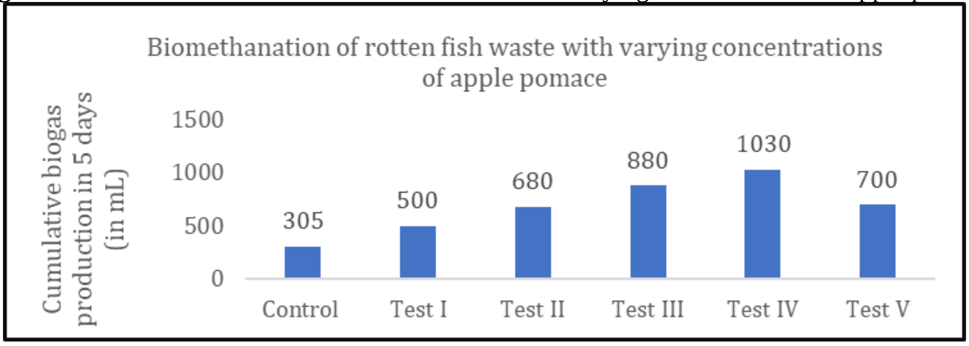

Table 4.17. Bio-methanation of rotten fish waste with varying concentrations of apple pomace

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste +60g fresh cow dung + 1%(w/v) apple pomace, made up to 600mL with tap water	500	63.93%
Test II	30g fish waste +60g fresh cow dung + 2%(w/v) apple pomace, made up to 600mL with tap water	680	122.95%
Test III	30g fish waste +60g fresh cow dung + 3%(w/v) apple pomace, made up to 600mL with tap water	880	188.52%
Test IV	30g fish waste +60g fresh cow dung + 5%(w/v) apple pomace, made up to 600mL with tap water	1030	237.70%
Test V	30g fish waste +60g fresh cow dung + 7%(w/v) apple pomace, made up to 600mL with tap water	700	129.5%

Supplementation of grape pomace and apple pomace as carbon supplements was done and it was found that the addition can effectively increase the biogas production when compared to control. The optimum concentration for maximum production of biogas was found to be 5% (w/w) for both apple pomace (Fig 4.18) and grape pomace (Fig 4.20). It is evident from Table 4.17 and Table 4.19 that the cumulative biogas production using apple pomace and grape pomace was 1030mL and 1050mL respectively.

Supplementation of grape pomace (carbon-nitrogen optimization)

Figure 4.19. Supplementation of grape pomace (carbon-nitrogen optimization)

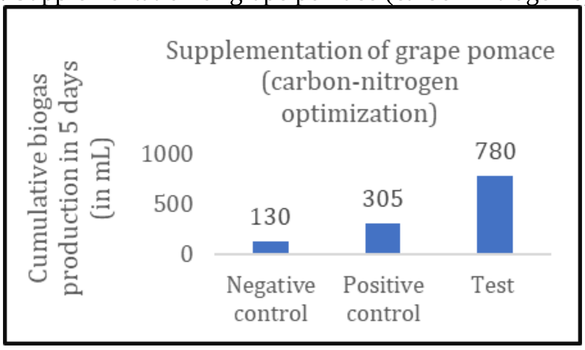

Table 4.18. Supplementation of grape pomace

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to positive control
Negative control	60g fresh cow dung made up to 600mL with tap water	130	
Positive control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test	30g fish waste +60g fresh cow dung + 1%(w/v) grape pomace, made up to 600mL with tap water	780	155.73%

Bio-methanation of rotten fish waste with varying concentrations of grape pomace

Figure 4.20. Bio-methanation of rotten fish waste with varying concentrations of grape pomace

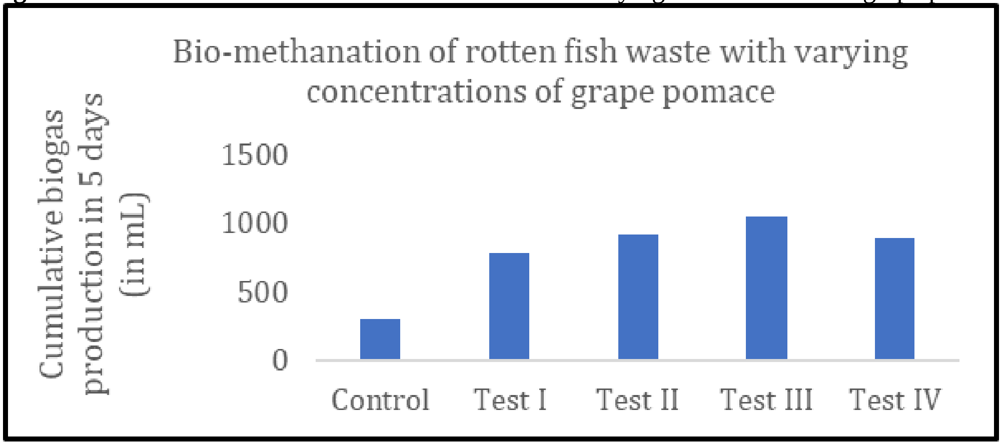

Table 4.19. Bio-methanation of rotten fish waste with varying concentrations of grape pomace

	Fermentation mixture	Cumulative production of biogas over a period of 5 days (in mL)	Percentage increase with respect to control
Control	30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	
Test I	30g fish waste +60g fresh cow dung + 1%(w/v) grape pomace, made up to 600mL with tap water	780	155.73%
Test II	30g fish waste +60g fresh cow dung + 3%(w/v) grape pomace, made up to 600mL with tap water	920	201.63%
Test III	30g fish waste +60g fresh cow dung + 5%(w/v) grape pomace, made up to 600mL with tap water	1050	244.26%
Test IV	30g fish waste +60g fresh cow dung + 7%(w/v) grape pomace, made up to 600mL with tap water	890	191.80%

Determination of methane content in biogas with rotten fish waste as substrate

Figure 4.21. Determination of methane content in biogas with rotten fish waste as substrate

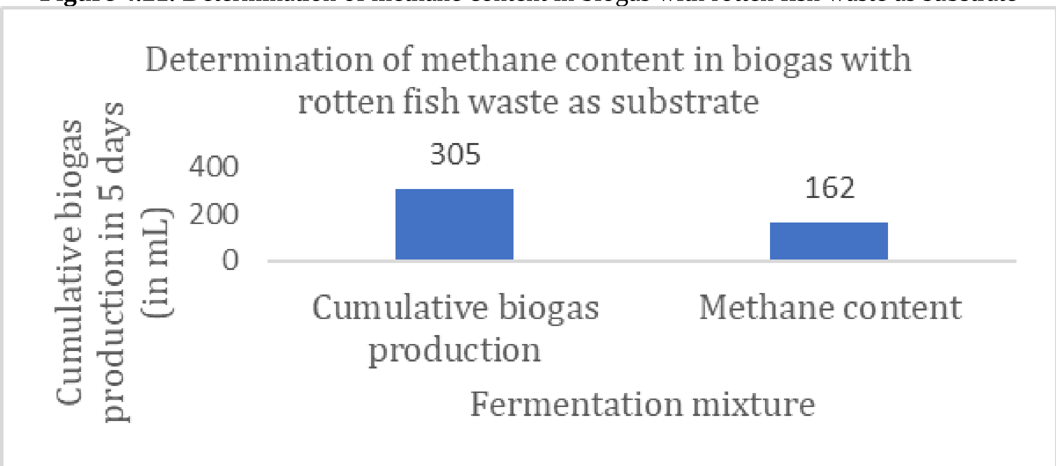

Table 4.20. Determination of methane content in biogas with rotten fish waste as substrate

Fermentation mixture	Cumulative biogas production	Methane content	Percentage of methane
30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	162	53.114%

Determination of methane content in biogas by the effect of coconut shell charcoal

Figure 4.22. Determination of methane content in biogas by the effect of coconut shell charcoal

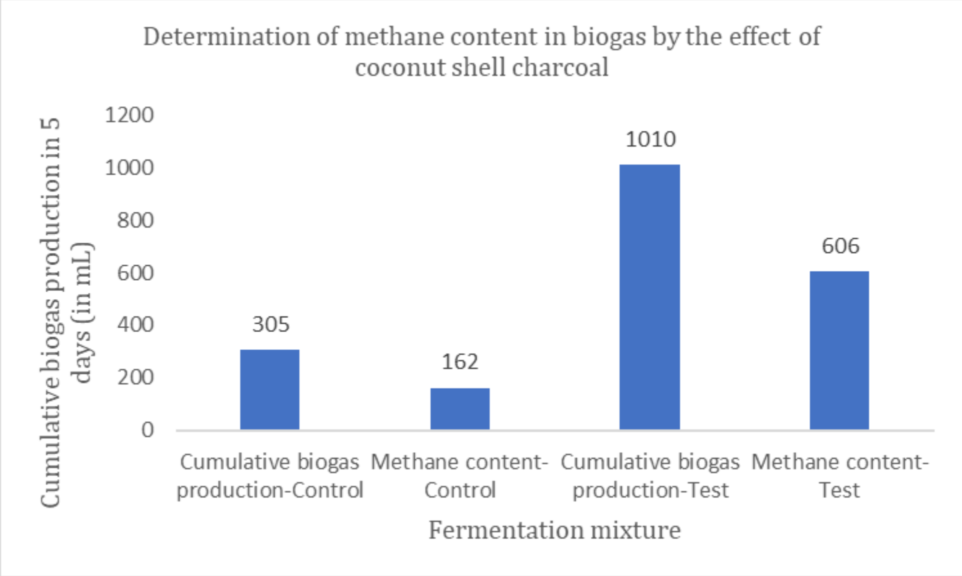

Table 4.21. Determination of methane content in biogas by the effect of coconut shell charcoal

Fermentation mixture	Cumulative biogas production	Methane content	Percentage of methane	Percentage increase of methane gas
30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	162	53.114%	
30 g fish waste+ 600mL water+ 60g cow dung+ 30g coconut shell charcoal (5%)	1010	606	60%	6.89%

Determination of methane content in biogas by the effect of apple pomace

Figure 4.23. Determination of methane content in biogas by the effect of apple pomace

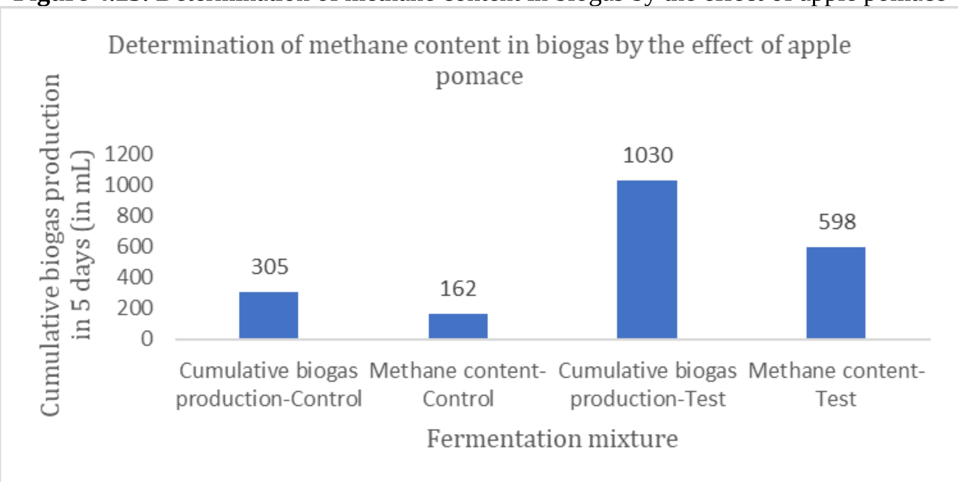

Table 4.22. Determination of methane content in biogas by the supplementation of apple pomace

Fermentation mixture	Cumulative biogas production	Methane content	Percentage of methane	Percentage increase of methane gas
30g fish waste with 60g fresh cow dung made up to 600mL with tap water	305	162	53.114%	
30 g fish waste+ 600mL water+ 60g cow dung+30 g apple pomace	1050	598	58%	4.89%

Biogas is a renewable fuel produced from the anaerobic digestion of organic feedstocks including municipal waste, farm waste, food waste, and energy crops. Raw biogas typically consists of methane (50–

75%), carbon dioxide (25–50%), and smaller amounts of nitrogen. Trace levels of hydrogen sulfide, ammonia, hydrogen, and various volatile organic compounds are also present in biogas depending on the feedstock. The amount of methane content in the biogas was determined using CO_2 scrubbing method using 5% NaOH. The methane content in the biogas was found to be 162mL which is 53.11% (fig 4.23). There is 6.89% increase in methane content in the experiment with 5% (w/v) coconut shell charcoal (fig 4.22 and table 4.21). Apple pomace is an excellent substrate for bio-methanation. There is a 4.89% increase in methane content in the experiment with 5% (w/v) apple pomace (table 4.22).

V. Conclusion

- Rotten Fish waste is an excellent substrate which can be channelized through bio-methanogenic pathway for the production of value-added products like biomethane.
- Addition of coconut shell charcoal and rice husk charcoal as carbon additives was found to be efficient for increasing the biogas production. The optimum concentration for maximum biogas production was found to be 5% (w/v) for both coconut shell charcoal (231%) and rice husk charcoal (221%). There was 6.89% increase in methane content with 5% (w/v) coconut shell charcoal compared to the control.
- The addition of metallic compounds like ferric chloride (68%), manganese chloride (159%) and cobalt chloride (192%) was found to be efficient for maximum biogas production. The addition of metal impregnated carbon additives was found to be excellent for higher biogas production. Activated carbon impregnated with compounds like Ferric chloride (60.31%), manganese chloride (58.33%) and cobalt chloride (56.92%) were found to very effective for enhancing biogas production.
- Supplementation of fermentation mixture with 5% (w/v) each of apple pomace (237.70% biogas yield) and grape pomace (244.26%) improved the production of biogas and the optimum concentration for maximum biogas production was found to be 5% (w/v). There was 5% increase in methane content in the experiment with 5%(w/v) apple pomace in comparison with control.

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