SUPPLEMENTARY MATERIAL

Simplicity Hits the Gas: A Robust, DIY Biogas Reactor Holds Potential in Research and Education in Bioeconomy

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Figure S1. (**a**) Valve connected to the biodigester with the respective rings and screw nut; (**b**) Bottle lid showing the hose pipe connected after glue (T-Rex Flex the universal adhesive sealant, Soudal).

Figure S2. - Filter system assembly schematic.

Figure S3. - Air chamber and the valve core.

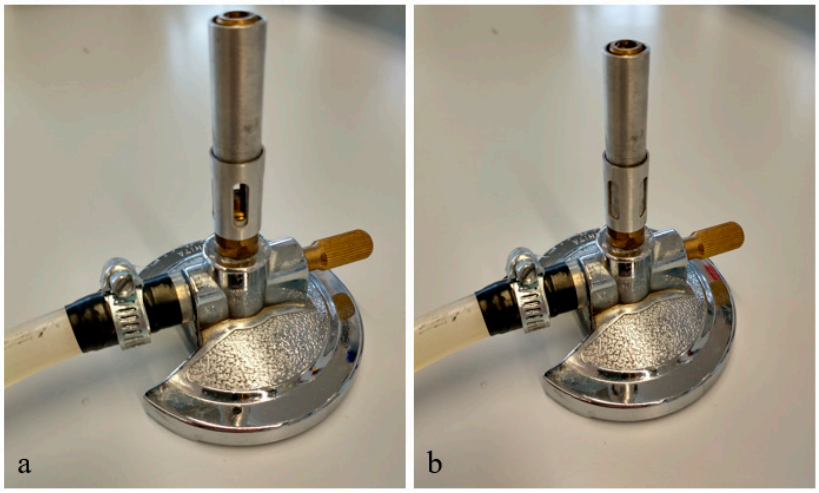
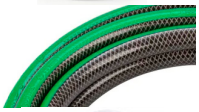


Figure S4. - Flame system with the air inlet open (**a**) and closed (**b**).

Figure S5. Color changing of NaOH solution during the absorption process.

Table S1. Material list and their respective pictures involved in the construction of the DIY biodigester.

Materials	Pictures
Water plastic bottle	
Plastic container	
Garden hose pipe	
Silicon pipe	
Ball valve tap	
Plastic closure valves	
T connector	
Nipple male-male	

Threaded sleeve female	
Female threaded hose connector	
Bunsen burner	
Binder clamp	
Glue (Soudal T-Rex Flex)	

Video. S1 https://we.tl/t-gblqSwpsz3 (https://www.youtube.com/watch?v=Jn2H2hiItos&t=6s).

Standard Operating Procedure (SOP) for DIY Biodigester

1. Purpose

The purpose of this SOP is to document the procedure for constructing and operating a DIY biodigester for research and educational activities.

2. Scope

The SOP provides guidelines on the design parameters, material selection, and step-by-step instructions for building a DIY biodigester. It details how to operate the biodigester, including feeding organic waste, monitoring gas production, and maintaining the system for optimal performance. The SOP also offers guidelines on safe practices during construction and operation, especially with regards to handling biogas and waste materials, as well as procedures for routine maintenance.

3. Applicability

This SOP is principally designed for individuals, educators, and researchers who are passionate about sustainable waste management, renewable energy, and hands-on educational activities. While the SOP is structured to align with educational environments such as schools, colleges, and research facilities, it emphasizes experiential learning. Though designed with universal applicability in mind, the SOP might require specific adjustments for regions with extreme weather conditions or high altitudes. Its focus is on DIY biodigesters, suitable for household or small community sizes, rather than commercial or industrial-scale operations.

4. Procedure

4.1. Materials and Equipment

- Fermentation Chamber:
  - 20-Liter recyclable plastic water bottle.
  - Valve with rubber rings (20 mm).
  - Appropriate glue (Soudal T-Rex Flex).
  - Hose pipe (16 mm, 2 meters).
- Filter System:
  - 500 mL plastic container.
  - 3M sodium hydroxide (NaOH) solution.
  - Thymolphthalein indicator.
  - Nipples, connectors, and sleeves (20 mm).
  - Appropriate glue (Soudal T-Rex Flex).
- Biogas Storage System:
  - Motorcycle air chamber (adapted).
  - Valve core removal tool.
- Flame Test System:
  - Bunsen burner.
  - Appropriate hose connectors.
- Other Equipment:
  - Plastic closure valves (16 mm).
  - T connector (16 mm).
  - Nipple male-male (32 mm).
  - Threaded sleeve female (32 mm).
  - Female threaded hose connector (32 mm).
  - Binder clamp.
  - Distilled water.

4.2. Assembly of DIY Biodigester

- Fermentation Chamber:
  - Take the 20-Liter plastic water bottle and create a 20-mm hole near the bottom for the valve using a drill.
  - Insert the valve through the hole and secure it with rubber rings for a tight fit.
  - Apply appropriate glue (Soudal T-Rex Flex) to all seals to prevent gas leakage.
  - Insert a hose pipe into the bottle cap, ensuring a snug fit for biogas flow.
  - Attach a T-connector before the filter for biogas sampling.
- Filter System:
  - Fill the 500 mL plastic container with a 3M sodium hydroxide (NaOH) solution.
  - Drill two 20-mm holes in the lid for connectors and nipples.
  - Secure the connectors, nipples, and sleeves to the lid, ensuring they have a tight seal by using glue.
  - Add thymolphthalein to the solution (e.g., mixing 5 ml 0.4 % thymolphthalein solution with 1 litre 3M NaOH solution).
- Biogas Storage System:
  - Adapt the motorcycle air chamber by using the valve core removal tool to allow biogas entry.
  - Ensure the chamber is sealed securely after adaptation.
- Flame Test System:
  - Attach the Bunsen burner to the piping system, ensuring the air inlet is closed for the flame test.
  - Test the Bunsen burner for proper function.

4.3. Operation of the DIY Biodigester

- Substrate and Inoculum Preparation:
  - Collect the substrate from a reliable source and store it appropriately (-4°C) in case you are not going to use immediately.
  - Substrate: Any type of organic matter (we recommend using buffalo manure as main source of inoculum and substrate)
  - In case you are going to use food waste, it is necessary to crush it with a food processor before to load the biodigester.
- Batch Experiments:
  - Set a 1:1 inoculum-to-substrate ratio (by volume) and a working volume of 12 liters.
  - For mono-digestion, use buffalo manure as the sole substrate, diluted with water in a ratio of 1:1.
  - For co-digestion, mix buffalo manure and food waste in a 1:1 ratio based and dilute with water in ratio 1:1.
- Digester Operation:
  - Add the prepared substrate and inoculum into the biodigester.
  - Close and seal the biodigester to maintain anaerobic conditions.
  - Place the DIY biodigester in an open place ensuring the contact with the sun light during the day.
- Mixing:
  - Manually agitate the contents of the biodigester twice a day.
  - Ensure thorough mixing to facilitate the anaerobic digestion process.
- Sampling:

        - Collect daily samples of 200 mL of biogas for subsequent analysis.
        - Periodically collect liquid samples for further measurements.
    - Monitoring and Record Keeping:
        - Record daily observations, including biogas production, temperature, and any notable changes.
        - Document any deviations from expected results.
    - Experiment Duration:
        - Continue the anaerobic digestion process for approximately 35 days or until biogas production becomes negligible.
        - Monitor the progress of the experiment regularly.

4.4. Safety Precautions

- Ensure proper ventilation during experiments.
- Wear appropriate personal protective equipment (PPE) including gloves and lab coat.
- Avoid direct contact with chemicals (NaOH) and be aware of their potential hazards.

4.5. Maintenance and Cleaning

- Regularly inspect the system for wear and tear.
- Clean and disinfect the fermentation chamber after each use.