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ORIGINAL ARTICLE

A sustainable and affordable production design of cleaner biogas from human excreta using eggshell

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Abstract

Low-quality, infectious pathogens, toxic chemicals, heavy metals, genotoxic, or radioactive particles are associated problems of biogas derived from human excreta. Most researchers had focussed solely on production optimization but with realities of pathogenic bioaerosols that may be dangerous to the users, there is a need to focus on the clean source-enhanced biogas production from human excreta. This research is designed to seek clear optimizable parameters in the clean production of biogas and its commercialization. A laboratory set-up was constructed to determine the biogas yield, bacteria mass, time of highest yield, and temperature. The surface response method was used to examine the influence of the microbial growth modifications from syntropic acetate oxidation (SAO) to a novel syntropic calcium acetate oxidation (SCAO) bacteria-using eggshell. It was observed that the human biogas production can be as high as 1020 g/ml on laboratory-scale within 30 days. More so, the biogas quality was improved by crashing the nitrogen gas content by 66%. The SCAO bacteria was observed to decompose the ammonia gas to form methane and NOx gases at a ratio of 3:1 within the first 20 days. The novel SCAO bacteria are suggested as good candidates to control ammonia and greenhouse gas emissions from human waste. It is recommended that there are still more to be done in culturing SCAO bacteria with slow growth and longer life span to make this process appreciate to industrial scale.

Keywords Biogas · Bacteria growth · Energy · Human excreta

1 Introduction

One of the reasons for projecting biogas from human excreta is its abundance. Its sustainability strictly depends on the growing population of human. In Africa, the population growth is on the positive trend. For example, Nigeria has a growing population rate of 3.2% annually. Currently, Nigeria is known as the country with the largest population in Africa. At this massive growing rate, it is expected that the amount of waste generated in Nigeria is also as equivalent as the individuals in the country. This situation in Nigeria is replicated all over Africa and most developing countries.

Biogas technology is at research levels globally as seen in the expansion of biogas systems in Sweden [1], anaerobic digestion of food waste [2], and biogas generation from wastewater [3]. For developed countries, there are more bioresources to explore. For developing country, the adoption of biogas from human faeces would be more feasible and sustainable due to the human population but its viability has been stunted over the years by corruption, insufficient funds, lack of dedication, ignorance, and laziness on the part of government and the people.

The basic allocation of energy resources in Africa is cooking and heating for different purposes. Biomass resources are the preferred candidates for this kind of activity. According to the global initiative on accessible, clean and efficient energy—sustainable energy for All (SE4ALL), very little progress has been made to provide access to non-solid cooking fuels since 1990. Nigeria is a vast country with a total of 356,667 mi^2 (923,768 km^2), of which 351,649 mi^2 (910,771 km^2 or 98.6% of total area) is land. This data means that adequate adoption of biomass resources should be more advantageous in the quest to solve energy

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crisis. This assumption applies to most developing countries. Among many challenges that has been identified above, technical know-how is the main challenge facing developing countries. This idea suggests that the way to solve Africa's energy crisis is to consider the most available energy sources with little technical input or skill to generate [4]. Biogas from human faeces was identified to be the most abundant and sustainable. Colón et al. [5] reported that 24 to 44 NL biogas can be produced per person per day. The population of Africa is a great asset in this regard and the perfection of the biogas generation from human faeces is a laudable idea that would solve Africa's energy problem via the adoption of standalone user option.

Like algae aerobic puns and palm oil effluent, human feces can be referred to as pending systems in biogas evolution [6]. The experimentation of Yacob et al. [7] showed that aside from the varying ambient condition of the biogas chamber, it was discovered that methane production was highest in regions where there is an excess accumulation of organic materials. Najafpour et al. [8] postulated that for the quality of biogas to attain the compressed natural gas (CNG), it must satisfy a percentage composition of CH_4, CO_2, H_2S, and H_2O to be $> 97\%$, $< 3\%$, $< 1\%$, and $< 2\%$, respectively. To achieve this feat, biogas from anaerobic co-digestion of two or more feedstock has been found to improve the quality and quantity of the resulting biogas. For example, Kuo and Dow [2] suggested the combination of two feedstock, i.e., food waste and municipal wastewater sludge,Mukumba et al. [9] used four feedstocks, i.e., human excreta, cow dung, and chicken manure,Ofoefule et al. [10] combined paper waste and cow dung. Adeyanju [11] gave a credible insight on the need to adjust the PH value in a co-digested feedstock. He proved that co-digested feedstocks (i.e., pig waste and cassava with wood ash) yielded better biogas quality and quantity than the mixture of pig waste and cassava peels only. This insight suggested that without the co-digestion process, biogas production can be improved using wastes that are likely to improve the quality and quantity of the biogas. It is in this light that eggshell was suggested. Eggshell is an available waste in the globe and its availability is assured when this project is implemented on a large scale.

Using human excreta for biogas in developing countries has its own disadvantage which is more social and environmental in context. The social context is the mindset of the users seeing the biogas from human excreta as a form of defilement when it is used for cooking or powering generators. Some cultural and religious attachments to human excreta signify uncleanliness; hence, the use of human excreta as a source of biogas may be an uphill task in this context. The environmental context illustrates a scenario that humans are potential carriers of diseases; hence, the emerging biogas from the human excretal are composed of harmful bioaerosols. Horváth et al. [12] noted that degradation of organic waste reduces the level of pathogens. This idea means that the reduced pathogens can be further reduced in the process. The adoption of eggshell in this process is scientifically proven as it possesses antimicrobial properties. Ohshima et al. [13] reported that eggshell has antimicrobial activity against bacterial vegetative cells, fungi, and bacterial spores. Hence, the introduction of eggshell powder in human excreta is expected to reduce the pathogens and improve methane production by influencing its pH value.

Many researchers had worked on the biogas from human excreta. It is generally noted that the biogas is of low quality and contains infectious pathogens, toxic chemicals, heavy metals, genotoxic, or radioactive particles [14, 15]. Current research on biogas derived from human excreta is geared towards solving the above challenge. Regattieri et al. [16], Colon et al. [17], Hadiyarto et al. [18], Yaradua, and Muhammad [19] and most researchers who had worked on biogas from human excreta had focussed solely on the optimization of biogas production through bioreactor design, co-digester, tunning atmospheric conditions etc. without considering other challenges as stipulated above. With the advent of pathogenic bioaerosols, there is the need to consider biogas optimization, as well as the reduction of the pathogenic concentrations.

In this study, the focus is to seek a sustainable method for the generation of biogas from human excreta with minimal concern for health and quality. The success of this research gives a ray of hope that some developing countries can solve most of their domestic energy needs based on financial viability and human population. This was achieved by the formation of a novel syntropic calcium acetate oxidation (SCAO) that have several advantages not limited to this study alone.

2 Design of human excreta biogas collection chamber

This section explains in detail the design set-up of the biogas proposed. This section also describes in detail the mode of material selection and the reasons behind certain materials. It further discusses the design and project setup of the individual materials to get achieve the project overall. [20] gave parameters for an efficient biogas digester, i.e., anaerobic environment, temperature, pH, type of substrate, substrate size, C/N ratio, ratio (F/M), and a substrate with microbes. Colón et al. [5] had suggested that efficient biogas digester should have improved sanitation, sterilization stages and possibly post-treatment unit to reduce VFAs to lower concentrations. The major unit of a digester is the degradation process. The degradation process in a biogas digester is in four phases namely hydrolysis, acidogenesis, acetogenesis,

and methanogenesis. Adeyanju [11] reported that each of the individual phases in the degradation process requires different groups of anaerobic microorganisms. For example, the hydrolysis phase requires microbes/hydrolytic bacteria to convert insoluble complex organic matters into monomers. Extensive work has proven that microbes' manipulations in each of the degradation processes can optimize biogas production [21].

In our design, we envisaged that the cost of biogas digester may be very expensive for users in developing countries. Hence, we seek a biogas digester that would be adequate for this purpose. Effluent et al. [22] gave a lofty biogas plant design as presented in Fig. 1. Since one of the objectives of this research is to seek a cost-effective biogas plant in developing countries. The effluent model was modified for buildings that have a minimum of thirty occupants. This plant can be modified by adding an optimization and treatment unit as presented in Fig. 2. The modified biogas plant may be affordable for schools in both rural and urban communities in Africa. The second design is the common design for the ordinary standalone user as presented in Fig. 3. The type of toilet system for this expedition is a latrine toilet. NDP [24] reported over 13 million users of latrine toilets in Nigeria. In other words, solving the energy demands of over thirteen million people in Nigeria is a great feat. Looking at sub-Saharan Africa, where over 60% of the population use a different form of latrine toilets as presented in Fig. 4, this deep state of poverty can be converted to opportunity using easily accessible waste material to boost biogas production for cooking and fueling generators. In this case, the system requires an additive that would solve the individual unit function in a biogas digester. The eggshell was reported to remove SO_2 and H_2S gas [25]. This makes eggshells have a trio function i.e., PH stabilizer, antimicrobial agent, H2S gas remover.

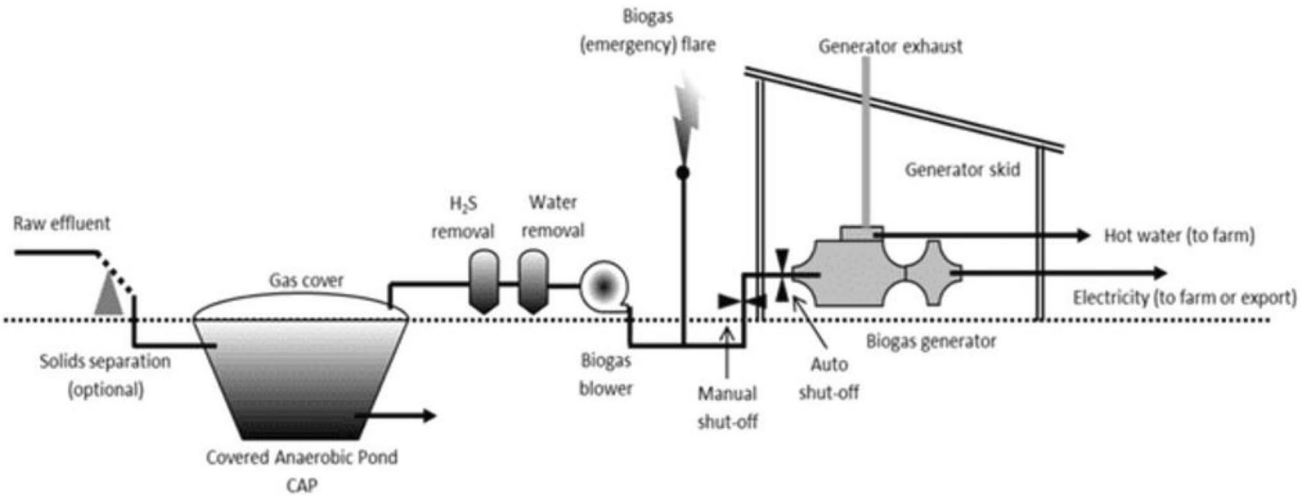

Fig. 1 Typical biogas piggery plant [22]

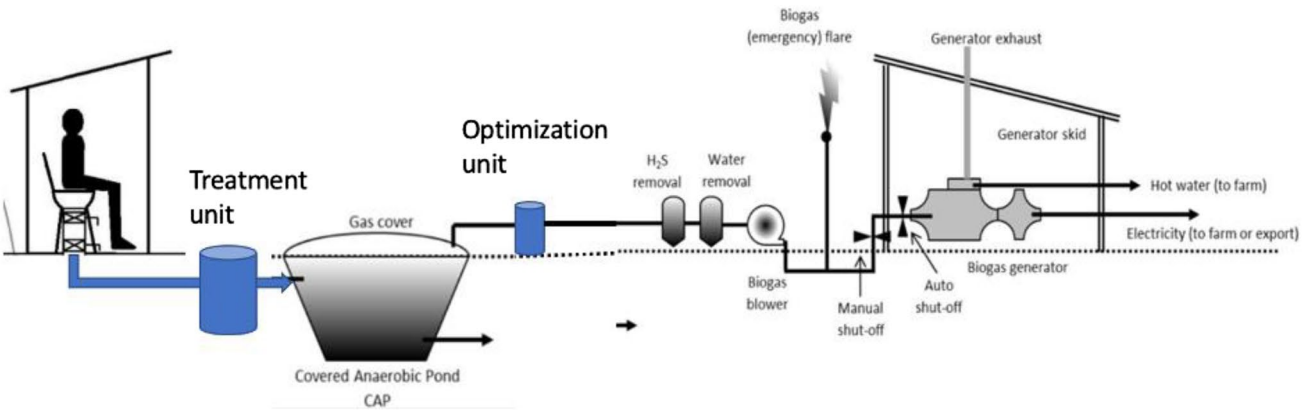

Fig. 2 Modified human excreta biogas plant [23]

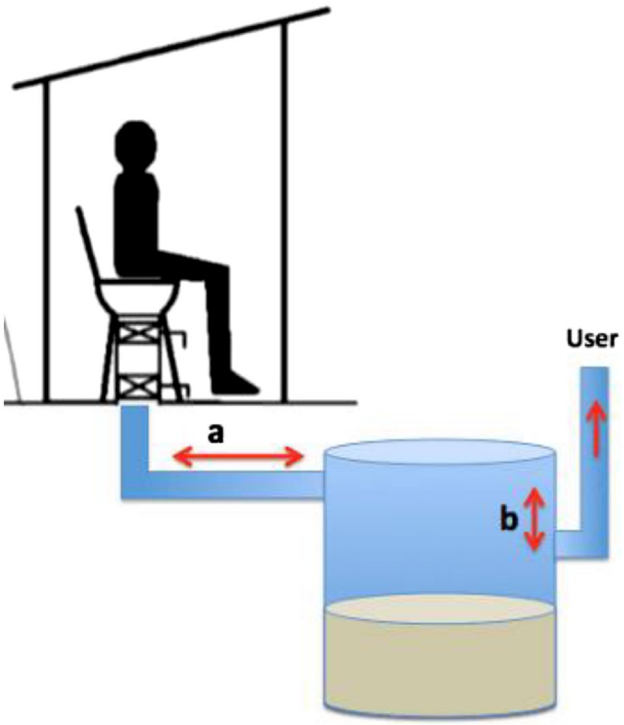

Fig. 3 Modified human excreta biogas plant

3 Current advances and limitations of cleaner biogas production from human excreta

Human excreta are the digested or absorbed food in the small intestine of humans. The human excreta are somewhat complex based on the nutrition lifestyle of the primary sources. The gas that emanates from the human excreta includes methyl sulphides, benzopyrrole volatiles, hydrogen sulphide which is a result of the food, herb, and drug intake of the individual. The main pathogens in the human system include Bacteroides species, Salmonella and Shigella, Yersinia, Campylobacter, Aeromonas, Candida, E. coli, Cryptosporidium, and Entamoeba histolytica. It is expected that this factor determines the volume and quality of biogas production. The challenges of biogas production are mainly the breaking down of the organic solid content and the reduction of pathogens. Recall that the reduction of pathogen in human waste had been briefly discussed earlier. The breaking down of the organic solid in human excreta is accomplished in the hydrolysis phase.

During hydrolysis complex, insoluble substrate such as polysaccharides are hydrolysed into smaller units by a large number of hydrolytic microorganisms (Clostridia, Micrococci, Bacteroides, Butyrivibrio, Fusobacterium, Selenomonas, Streptococcus) secreting different hydrolyzing enzymes such as cellulase, cellobiase, xylanase, amylase, protease, and lipase [26]. Many researchers have propounded laudable ideas to aid

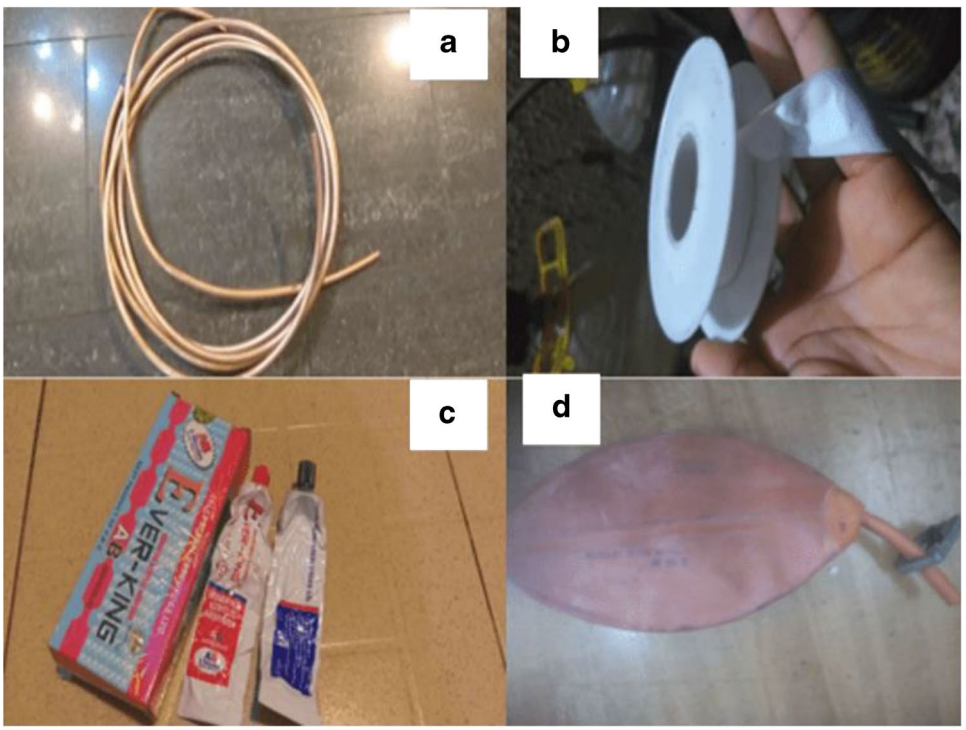

Fig. 4 Material for laboratory preparation. **a** Copper pipes. **b** Thread tape. **c** Four-min glue. **d** Bladder bag

the hydrolysis of complex biomass but in this case, the organic content of human excreta. Hadiyarto et al. [18] proposed the use of microbes for breaking down of complex compounds, i.e., the addition of the rumen from cattle dung to speed up the decomposition time in the hydrolysis process. Heubeck and Craggs [27] proposed the use of thermal treatment as a means to speed up the rate of hydrolysis. Emetere and Adesina [28] have confirmed these ideas in an experiment where biogas in sewage lines are measured at different times of the day or at different seasons in the year. Chulhwan et al. [29] did a comparative study on the thermochemical or biological hydrolysis. They observed that thermochemical hydrolysis showed better results than biological hydrolysis as the total chemical oxygen demand (tCOD) reduction is 88.9% and 79.5%, respectively. Big energy companies are using engineered enzymes such as the fungus Trichoderma reesei for the enzymatic hydrolysis process [30].

A novel study on the use of eggshell to speed up the hydrolysis process of banana pulp was first reported by [31]. The detailed analysis of the eggshell is presented in Table 1 below.

The use of egg-shell in the processing of human excreta to yield biogas is germane as the results of this study would enhance the clean and massive production of biogas from human waste.

4 Material and methods

The material selection for this particular project was done considering the following factors, i.e., the nature of feedstock to be analyzed, availability, the total cost of materials, and impact on the environment.

4.1 Construction of laboratory-scale biodigester

Figure 3 was therefore tested on a laboratory scale. The biogas laboratory setting was made up of two testing chambers. These two testing chambers each have a certain mass of human excreta and water mixture put in them. Each container has a copper pipe and a bladder connected to it for gas collection. The containers are designed such that they are airtight sealed with thread tape at the top and glue is used to hold the pipe in place properly.

The laboratory-scale biodigester that was used for this experiment was constructed using common material as described in Fig. 4. A copper pipe of the radius of 15 mm was used. This material was chosen in order to allow the steady flow of the gas produced to its endpoint. This copper was cut and bent in a *U* shape to accommodate the size of the containers. They were attached to the wood in order to be held down properly. Adhesive gum and thread tape were used to seal the threaded cover of the container to block any unseen space to avoid leakage of the biogas and to maintain an anaerobic state. This thread tape was wrapped numerous times around the cover with no particular length. The adhesive gum was used to hold the wood and pipe firmly together. A 250-ml bladder bag was used to collect the gas from the reaction process in Fig. 5. Four parameters were measured during this experiment, i.e., the ambient temperature in the laboratory, the mass of the biogas that is trapped within the balloon, the bacteria growth as seen in the increase of the digestate mass, and the time which was measured in 20 days.

The construction of the laboratory bioreactor gave a clear indication that material selection is salient in the construction of a low-cost bioreactor. On the first attempt at the

Table 1 Properties of digester content of un-calcined eggshells [31]

Reactor	C_0	C_1	C_2	C_3	C_4	C_5
Eggshells (g/L)	0	1	3	5	7	9
Initial pH	5.92 ± 0.04	6.05 ± 0.05	6.38 ± 0.06	6.41 ± 0.07	6.54 ± 0.05	6.71 ± 0.07
Final pH	4.46 ± 0.03	4.80 ± 0.04	5.31 ± 0.05	5.68 ± 0.04	6.01 ± 0.06	6.52 ± 0.05
Initial COD (g/L)	13.75 ± 0.9	17.84 ± 1	15.62 ± 0.8	19.21 ± 0.1	20.43 ± 0.7	24.11 ± 1
Final COD (g/L)	6.13 ± 0.2	7.65 ± 0.5	6.1 ± 0.1	7.2 ± 0.6	6.74 ± 0.2	7.1 ± 0.6
% COD removal	55.4	57.1	60.9	62.5	67.0	70.6
Initial TS (g/L)	15.4 ± 0.2	17.84 ± 1.2	19.02 ± 0.6	18.42 ± 0.8	19.72 ± 0.16	18.59 ± 1.7
Final TS (g/L)	5.5 ± 0.7	6.33 ± 0.4	5.65 ± 0.3	1.82 ± 0.06	1.49 ± 0.06	1.06 ± 0.1
% TS removal	64.0	64.5	70.3	90.1	92.4	94.3
Initial VS (g/L)	3.51 ± 0.7	3.55 ± 0.52	4.86 ± 0.4	4.79 ± 0.7	5.55 ± 0.1	5.23 ± 0.9
Final VS (g/L)	2.45 ± 0.5	2.17 ± 0.52	2.55 ± 0.2	2.1 ± 0.1	2.56 ± 0.3	1.84 ± 0.2
% VS removal	30.1	38.8	47.5	56.2	59.3	64.9
Initial VFA (g/L)	0.113 ± 0.02	0.057 ± 0.002	0.053 ± 0.001	0.052 ± 0.001	0.050 ± 0.0005	0.032 ± 0.0001
Final VFA (g/L)	0.169 ± 0.002	0.086 ± 0.001	0.08 ± 0.0004	0.078 ± 0.0002	0.075 ± 0.0001	0.048 ± 0.0002

COD chemical oxygen demand, *TS* total solids, *VS* volatiles solids, *VFA* volatile fatty acid

Fig. 5 Laboratory set-up of common biogas collection from human waste

construction, a wine bottle and balloon were used but there were leakages despite all efforts to curb them. When the system was further improved but the balloon got weak and exploded. Success was later achieved using the materials described above.

4.2 Material screening

In this section, the material used for the experiment was discussed. The eggshell was used as the optimizing agent for improving the quality of the biogas production from human excreta. The eggshell was gotten from chicken poultry. The eggshell is the outer covering or hard external layer of an egg. The eggshell consists of calcium carbonate. The protein content in this eggshell has an important role to play in the strength of an eggshell. This calcium carbonate actively reacts when put with acid to form carbon dioxide. The structure of the eggshell has been known for more than a century. Chemical and histochemical methods have been used to investigate the composition of the shell membranes. Through this process, it has been established that the membranes are largely made up of protein due to their high content of cysteine. It has also been seen to contain keratin. The presence of small amounts of hexosamine, galactose, mannose and traces of sialic acid. According to chemical analyses, the eggshell is made up of 97% calcium (Table 2). The eggshell is lined with two membranes each of which is made up of a network of fibres that are thick [32].

The raw egg was washed with fresh water before breaking the shell. This process was done to remove external contaminants and the eggshell was left to dry using open-air drying. The dried eggshell was ground to powder as presented in Fig. 6.

Table 2 Percentage elements contained in eggshell [33]

Elements	Weight percentage Wt%
CaO	76.992
K_2O	0.0542
MgO	0.9261
Na_2O	0.1046
C	21.1286

4.3 Microbes screening

The mixing ratio of the slurry of fresh human excreta was 1:5. The microbe was cultured for 15 days. There are trillions of microbes that reside in the human excreta [34]. Microbes are microscopic organisms with 4 growth phases consisting of adaptation phase, growth phase, stationary phase, and death phase [18]. At day 15, it is expected that the microbes are at the stationary phase. One hundred milliliters of the sludge was used for the experimental work.

4.4 Technical screening

The mixing ratio of the slurry of another batch of fresh human excreta was 1:5. Two different containers were labelled A and B. Containers A and B are made up of human excreta whose slurry gave a total mass of 2325 g. One hundred milliliters of the cultured microbes were added to the slurry of both containers. Four hundred thirty-four grams of the powdered eggshell was added to the slurry in container B, i.e., making the total mass as 2759 g. Hence, the ratio of eggshell to excreted was roughly 1:5. Each container was separately sealed weighed and connected to individual pipelines to allow the

Fig. 6 Crushed eggshell

respective collection of gases. These containers are subsequently monitored for pressure building via opening the tap lock due to the nature of the material of the container being used (plastic). The duration of this experiment span a month. In the first 2 weeks of this setup, no significant gas formation was recorded. The bladder remained flat throughout. During the third week of the setup, the bladder bag was half blown. On the third week, the bladder bag was fully blown. During this process, no external forces like shaking or heating were carried out in this experiment. After the third week, the gases supply in the set-up continued to grow exponentially. The biogas was measured accordingly by weight and component.

The biogas that was measured was the ones trapped in the 250 ml bladder. The daily biogas volume (V_B) was generated using the formular described by Yaru et al. [35]:

$$R_0 = \frac{R}{M} \times \%\ composition \tag{1}$$

$$V_B = \frac{R_0 + T_0}{P_0} \tag{2}$$

where *Ro* is the specific gas constant of a gas (J/kg K), *R* is the universal gas constant (J/kg K), *M* is the molecular mass of the gas concerned, *Po* is the estimated daily pressure of the digester, *To* is the estimated daily temperature in °C of the digester and *V* is the volume of biogas generated in m^3. The biogas yield was calculated using the formular presented by Olojede et al. [36].

The biogas yield was calculated via

$$Biogas\ Yield = \sum \frac{V_{BN}\ (Nml)}{Mass\ (g)} \tag{3}$$

V_{BN} is the normalized amount of biogas volume and was estimated via:

$$V_{BN} = V_B \times F \tag{4}$$

F is the gas factor and it is calculated by the formular

$$F = \frac{\left(P - P_{H_2O}\right) \times T_0}{(t + 273.15) \times P_0} \tag{5}$$

where $To = 273.15$ K (standard temperature), t = gas temperature in °C, $Po = 1013.25$ mbar (standard pressure), and P = air pressure, P_{H_2O} was calculated using Omni [37].

4.5 Measuring instruments

Each of the gas samples produced from the experiment was analysed in the laboratory using the RASI700 BIO Portable Gas Analyzer. Some of the readings are in ppm (part per million) while some are in % vol (percentage volume). The control experiment (pure human excreta) was measured using gas chromatography (GC–MS), i.e., Finni-gan Focus GC, ITQ 700, Thermo Electron Corp. The standard as highlighted in Knízek et al. [38].

5 Theory and calculation

The mixing ratio of the slurry can be calculated using Eq. 1 while the biogas production can be calculated using Eq. 2 [19].

$$V_d = (B + W)R_t \tag{6}$$

R_t is the retention time in days, V_d is the volume of the bladder bag (ml), *B* is the biomass (kg), and W is the volume of water (ml).

$$G = G_s \times V_f \tag{7}$$

V_f is the weight of feedstock, G_s is the gas yield, G is the biogas production. The assumed energy content of the produced biogas (E) as [39]

$$E = G \times 0.006 \frac{kWh}{dm^3} \tag{8}$$

or

$$E = G \times 21.6 \frac{kJ}{dm^3} \tag{9}$$

Based on the above equation, some software have been developed to calculate biogas production per year, electricity production per annum, thermal energy production per annum, total digestate gotten per annum, approximate inert contaminants, greenhouse gases produced, electricity revenue in dollars, and plant cost estimation [40]. The percentage ratio of the eggshell content was optimized using varying weight percentages of agricultural waste that range from 10%, 20%, 30%, and 40%.

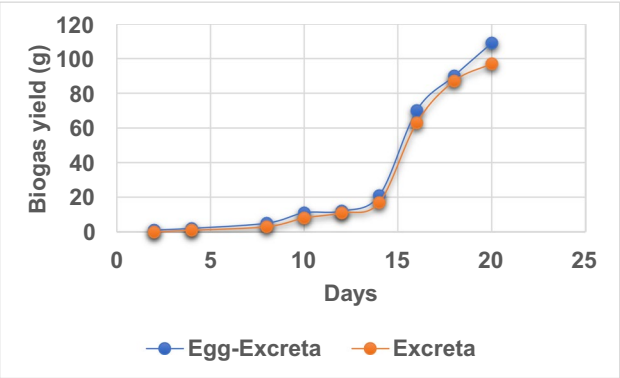

Fig. 7 Biogas yield from both containers

6 Results and discussion

The biogas yield (in grammes) from container B (egg-shell and excreta) and container A (pure excreta). The biogas yield in container B was seen to have a higher biogas yield than container A. This fact because the biogas yield becomes more distinct as the duration of digestion is extended (Fig. 7). This result supports the idea that biogas yield will be higher in a slur that has high organic contents. It was observed that at the initial stages of degradation, that is, between the first 15 days, the biogas yield in both containers (i.e., A and B) were almost the same. As the degradation progresses, the biogas yield from container B becomes distinct. This result is because the main content of the egg, that is, calcium carbonate plays a major role in oxidizing the degradation process. This result simply means that as the syntropic acetate oxidation (SAO) plays an important role in methane formation in biogas yield [41], the calcium carbonate in the eggshell is further broken down to oxidize the anaerobic process and modify the SAO bacteria to syntropic calcium acetate oxidation (SCAO) bacteria (Fig. 8).

The modified SCAO bacteria naturally have an enhanced growth as the calcium in its new component enhances the growth of a wall-less, L-form of the bacteria [42]. This fact is further proven in the biogas yield projections from day 20 to 30 as shown in Fig. 9. It is observed that the SCAO bacteria become more active as the day progresses. Figure 9 shows that the biogas yield will increase from 100 g to 2 kg. This insight is very crucial in the estimation of biogas production in Fig. 10 from Eq. (7). This result means that if

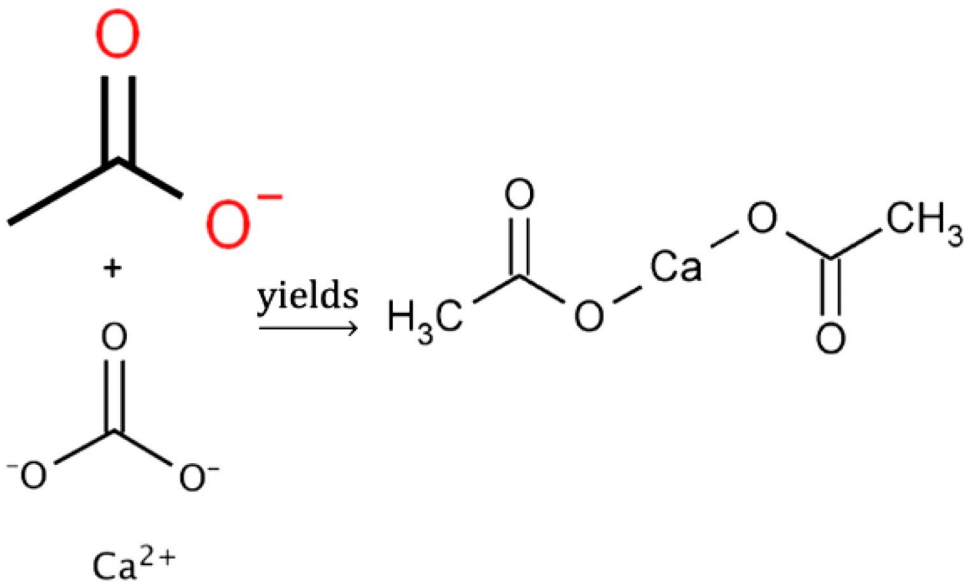

Fig. 8 Microbial modification of the SAO organism

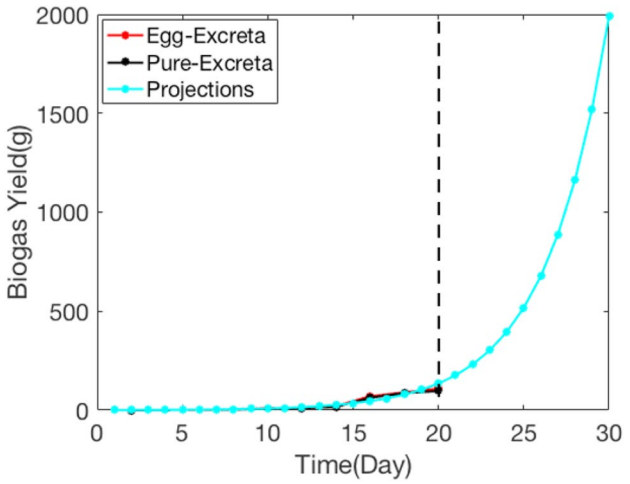

Fig. 9 Biogas yield projection beyond the 20th day

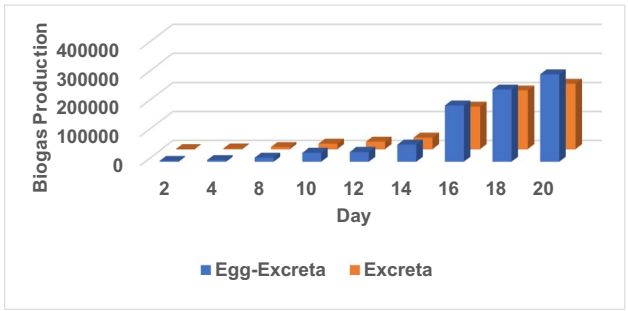

Fig. 10 Biogas production in laboratory scale

this process is scaled-up to industrial production, i.e., using the biomass weight ratio, there is huge potential that a high quantity of biogas is achievable. It was seen that the trend of the biogas production follows an exponential relation shown in Eq. 10.

$$y = 0.6184e^{0.2692x} \tag{10}$$

The quality of the biogas was characterized to determine the percentage constituents of the biogas yield. The absorption spectroscopy reveals that the pure biogas from human excreta was mainly nitrogen, methane, and carbon dioxide (Fig. 11) in the ratio 23:15:1 as shown in GC–MS analysis. From the above results, there are more chances that ammonia gas formation is high in biogas content from human excreta. This fact is supported by the iso-butane (0.02%), N-pentane (0.02%), and propane (0.01%) in the gas (Fig. 11). These gases may disintegrate at further biodegradation to form ammonia. Though this fact may vary in human excreta which is as a result of the type of food consumed by the individual or underlying ailment in the individual.

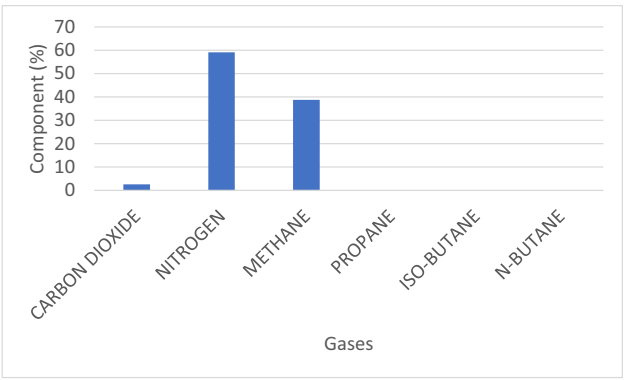

Fig. 11 Percentages of elements present in biogas using a GC–MS

Table 3 Comparative percentages of elements present in biogas

	Pure_Excreta	Egg_Excreta
Nitrogen	59	20
Methane	11.6	12.74
Carbon dioxide	2.3	9.8
Oxygen	0.8	3.1

The comparative analysis of the biogas quality in experiment/containers A and B is presented in Table 3. This result shows that the nitrogen content reduced by 66% which means that there most nitrogen was converted to NOx gas. The methane quality dropped by 68%, while the carbon dioxide content increased by 78%. Also, there was an introduction of oxygen content which amounted to 0.08% of the constituents in experiment/container B. These results further illustrate the role of the SCAO bacteria in enhancing biogas quality. The SCAO bacteria were found to demonstrate significant growth as seen by the weights of the container at the beginning and end of the experiment. It was observed that the weights in container B had increased from 2325 to 3042 g. This shows that the additional masses are known as bacteria mass for knowing bacteria or microorganism population within a system. Senés-Guerrero et al. [43] had earlier characterized such residue to be bacteria (82–88%) and Archaea (8–15%) at each stage of biogas production. However, as the duration of the degradation increases, the SCAO bacteria decompose the ammonia gas to form methane and NOx gases at a ratio of 3:1 within the first 20 days. This feat is laudable as scientists have shown that lignin, cellulose, and hemicellulose are components of the human excreta [44]. The selective degradation of lignin, cellulose, and hemicellulose by SCAO in human excreta leads to saccharification that would lead to further biogas generation. This fact is evident in the conversion ratio that had increased to 6:1 by the 30th day. The novel SCAO bacteria are good candidates to control ammonia and greenhouse gas

emissions from human waste. The experiment was scaled-up to understand the activities of the microbial growth using the response surface method.

The central composite design with two level 2 factors was adopted. The biogas yield was computed, i.e., considering salient parameters such as temperature, time, the mass of biogas, i.e., biogas yield, and a microbial population that is assumed from the increase in the mass of digested. Literature shows that at a conducive environment, there is an exponential increase in numbers or bacterial mass that is directly proportional to time [45, 46]. Hence, the behavior of the system is explained by the following second-order polynomial model which was performed for 20 runs.

$$Y = c_0 + c_1 y_1 + c_2 y_2 + c_{11} y_{12} + c_{22} y_{22} + c_{12} y_1 y_2 \quad (11)$$

Y is the predicted response, y_1 is the input variable for temperature, y_2 is the input variable for the mass of biogas, y_3 is the input variable of microbial population, c_0 is constant, y_1, y_2, is the linear coefficients, y_{22} is the quadratic coefficient, and y_{12} is the cross-product coefficient.

The curve fitting analyses of the two-dimensional relation of the parameters are analyzed as presented in Figs. 12, 16, and 18. Figure 12 shows the biogas yield with respect to time. The upward trend of the positive parabolic curve is the first evidence that the process has the potential of increasing should the experiment extends beyond 30 days. The second and third-order was seen to be more adequate to describe its relationship. This result is the second evidence to show the prospect of this experimentation on a larger scale. Figure 13 presents the residual plot that is expected to affirm the polynomial order of the relation. The residual plot is the graphical technique that attempts to show the relationship between a given independent variable (time) and the response variable (biogas yield). The residual plot further affirmed that the mathematical relationship between the biogas yield and time is the second and third polynomial order. This result becomes the third evidence that there are prospects for optimizing the process for industrial-scale biogas production. Due to the ability of SCAO to convert high molecular weight compounds in the substrate into lower mass compounds that can enter the fermentation process, the growth pattern was investigated to mainly see its shortcoming. The growth pattern of the bacterial as seen in the increase in the mass of the digestate with time is shown in Fig. 14 below. Like the biogas yield, it was observed that the relation between microbial growth and time is best described in the second and third polynomial order.

The shape of the graph is in line with the bacteria growth pattern shown in Maier [45]. The logarithmic fit clearly shows that bacterial growth has reached its peak in 20 days. The residual plots further confirm that the mathematical description between the bacteria growth and time is in the second and third polynomial (Fig. 15). However, the residual

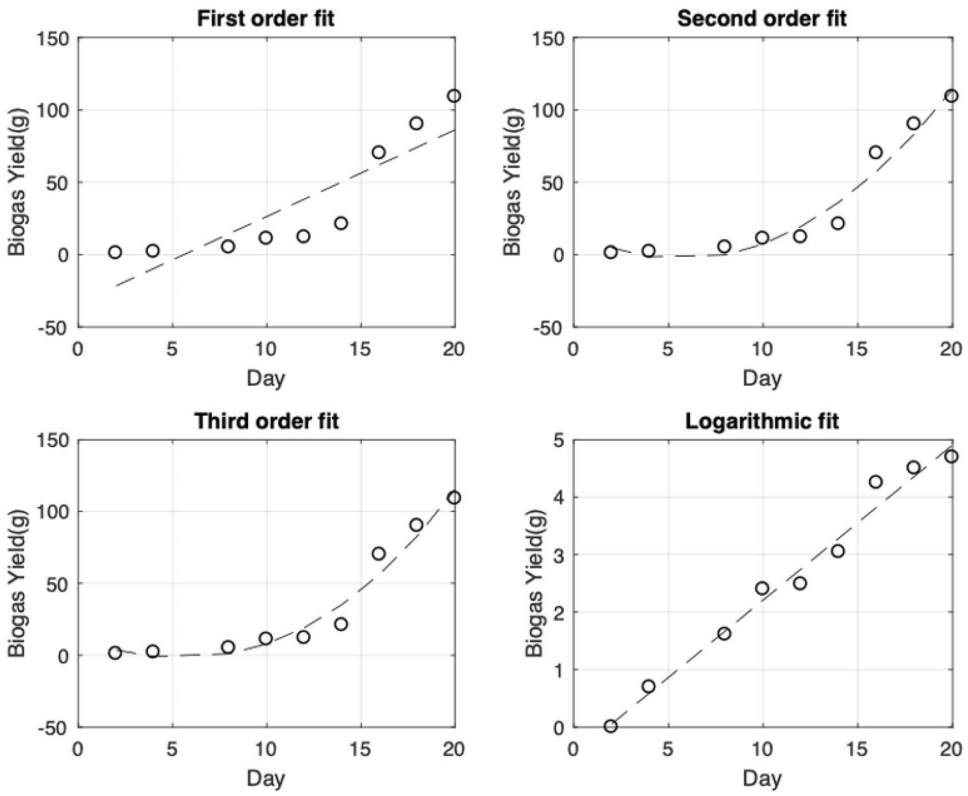

Fig. 12 Curve fitting between the biogas yield and time

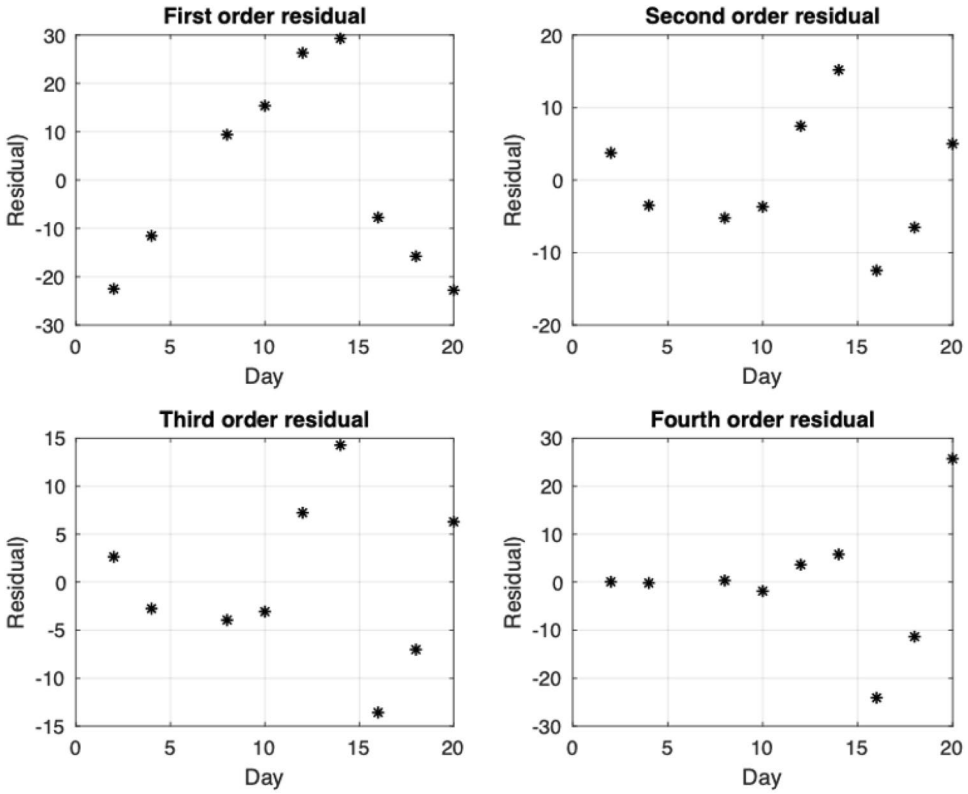

Fig. 13 Residual plot between the biogas yield and time

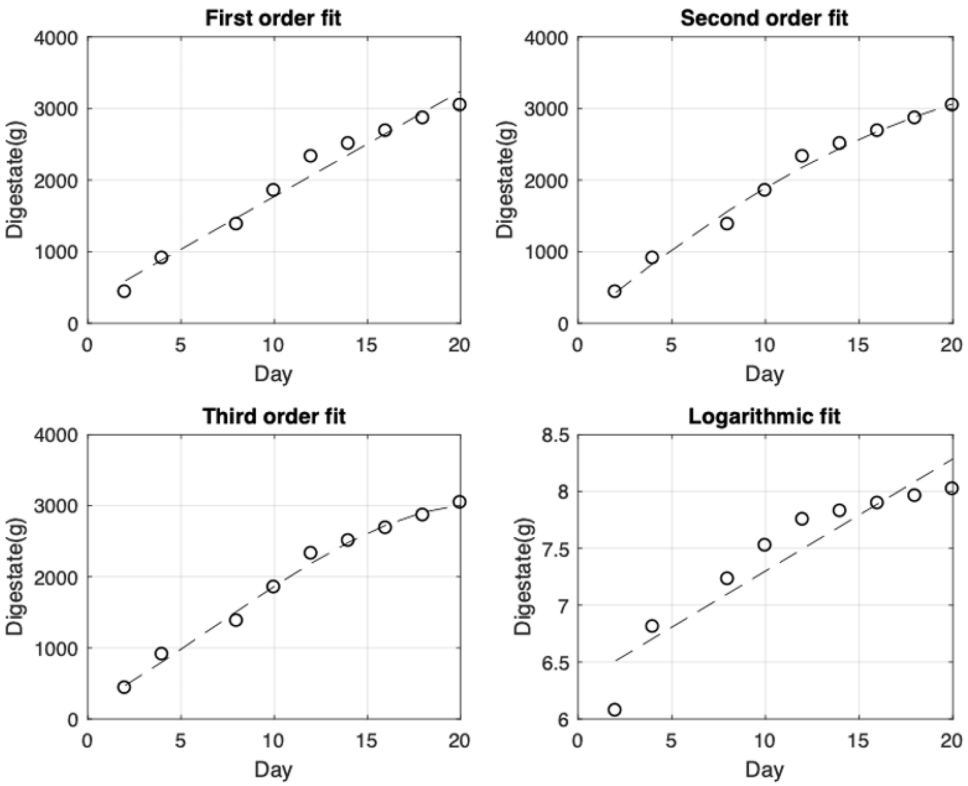

Fig. 14 Curve fitting between the bacteria growth and time

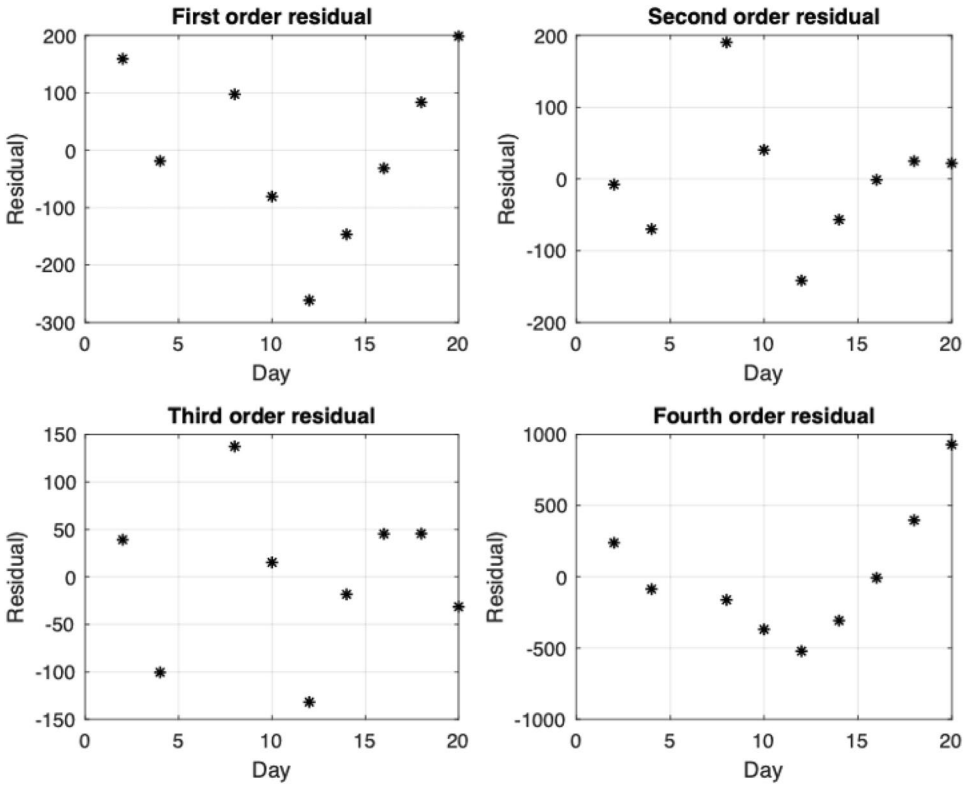

Fig. 15 Residual plot between the bacteria growth and time

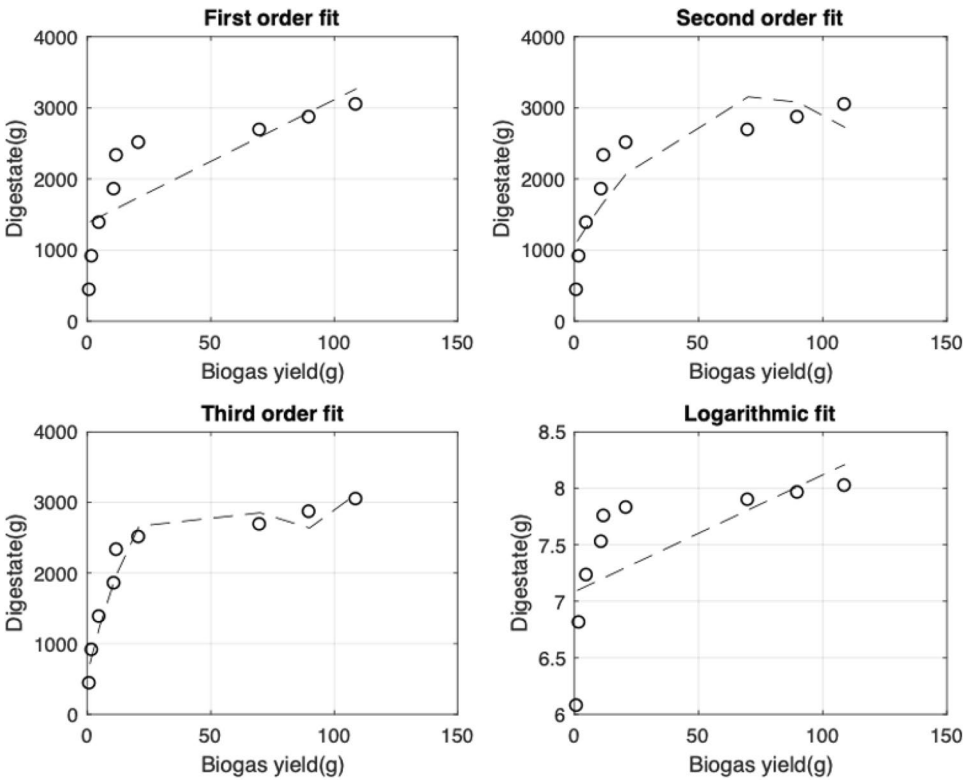

Fig. 16 Curve fitting between the bacteria growth and biogas yield

plot shows that the second-order polynomial describes both parameters. As described earlier, these evidence are indication that the project has prospects for expansion beyond the laboratory scale. The last relation that was investigated is the mathematical relation between the biogas yield and the bacteria growth as presented in Fig. 16. The graph shows

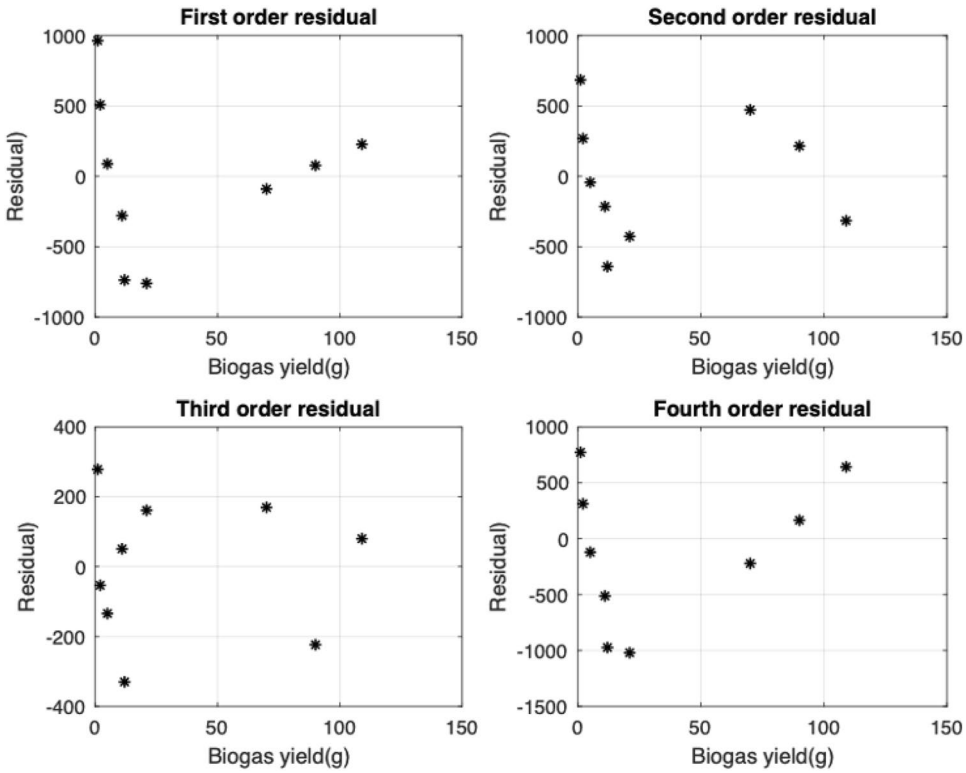

Fig. 17 Residual plot between the bacteria growth and biogas yield

Table 4 Statistical analysis of bacteria growth and biogas yield

Parameter	Mean	Std error	Stand dev	Coeff var
Biogas yield	35.70	14.03	42.08	117.99
Bacteria growth	1998.11	305.3095	915.93	45.83

Table 5 Correlation between bacteria growth and biogas yield

	Sum of sqrs	df	Mean square	*F*	*p* (same)
Rows	3.60822E06	8	451,027	1.157	0.4206
Column	1.73303E07	1	1.73303E07	44.47	0.0001577
Error	3.11735E06	8	389669		
Total	2.40559E07	17			

two-stage of occurrence, i.e., when the SCAO bacteria initiate growth and when it stabilizes growth. When the SCAO stabilizes its growth, more yield of biogas is expected at a shorter time. These results further strengthen the projection provided in Fig. 9. Figure 16 shows that the third-order polynomial can best describe the relationship between both parameters. More so, the residual plot shown in Fig. 17 also affirms that the third polynomial can describe the extensive nature of the project. The statistical analysis of both parameters (i.e., bacteria growth and biogas yield) is displayed in Table 3. The results displayed in Table 3 corroborated the study by Asikong et al. [47] that shows that there is significant variation ($p < 0.05$) in the anaerobic bacteria counts between the feedstock and duration of digestion. The individual analysis shows that an increase in the SCAO bacteria leads to an increase in the biogas yield. Also, the coefficient of variation that describes the statistical measure of the dispersion of data points around the mean shows that biogas yield may at a point not depend on the further increase of the SCAO bacteria to increase its magnitude. This result means that declining SCAO bacteria growth at some point would not be directly proportional to the biogas yield. The correlation between the bacteria growth and biogas yield was investigated as shown in Table 4. The high F-factor shows that the dataset is more correlated within its column. In general, no correlation was found between both parameters. This result supports the earlier fact.

The different coefficients displayed in Eq. 11 were determined using the regression analysis in Table 5. Three variations of Eq. 11 were investigated with the aim of determining the relationship with the highest regression parameters as shown in Table 5. The coefficient for each variation was obtained and presented in Table 6. The 3D patterns of the variation are displayed in Fig. 18. It was observed that the bacteria growth, temperature, and biogas yield did not provide expected result using Eq. 11. This idea is because the R^2 was found to be negative. Hence, the optimization process would work better with the first modification. The 3D illustration of the biogas yield as against bacteria growth and temperature clearly supports the fact that the two stages

Table 6 Regression analysis of Eq. 11 under certain conditions

Biogas yield as against bacteria growth and temperature				
Equation	R^2	Adjusted R^2	RMSE	Figure
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}y_1y_2$	−0.4958	−2.989	84.05	21a
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}y_1$	0.8999	0.733	21.74	21b
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}$	0.8883	0.7021	22.97	21c
Biogas yield as against bacteria growth and time				
Equation	R^2	Adjusted R^2	RMSE	Figure
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}y_1y_2$	0.9689	0.9171	12.12	21d
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}y_1$	0.9689	0.9171	12.12	21d
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}$	0.9689	0.9171	12.12	21d

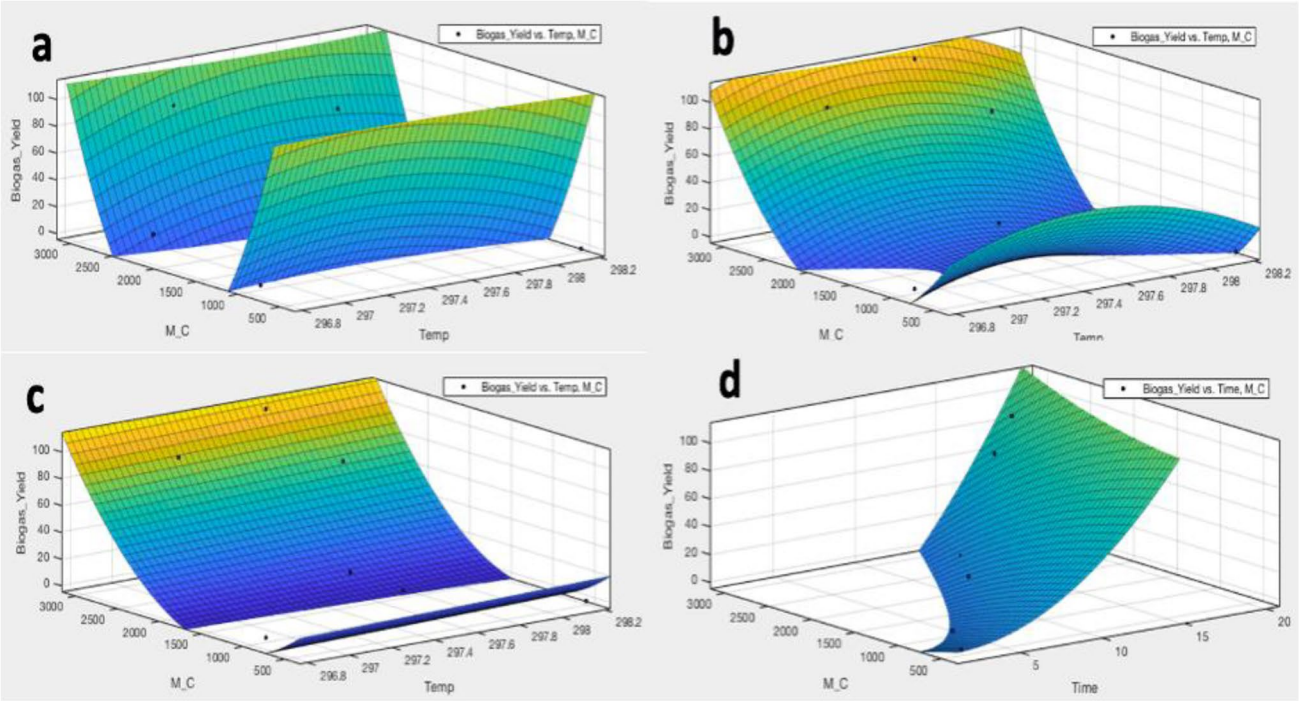

Fig. 18 3D pattern of variations in Eq. 11 for the determination of the best regression parameters

Table 7 Coefficients of Eq. 6 under certain conditions

Biogas yield as against bacteria growth and temperature						
Equation	c_0	c_1	c_2	c_{11}	c_{22}	c_{12}
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}y_1y_2$	3.338e+06	2.247e+04	−5.143	−37.82	0.0001105	0.01599
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}y_1$	−4.77e+06	−3.693e+04	−0.1252	−53.92	4.358e−05	6.901e+04
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}$	0.1631	−3.071	−0.07946	0.01075	3.29e−05	0.5454
Biogas yield as against bacteria growth and time						
Equation	c_0	c_1	c_2	c_{11}	c_{22}	c_{12}
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}y_1y_2$	8.418	−1.071	−0.01047	0.2152	−7.654e−06	0.3642
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}y_1$	8.418	−0.4536	−0.01047	0.5794	−7.654e−06	−0.6177
$Y = c_0 + c_1y_1 + c_2y_2 + c_{11}y_{12} + c_{22}y_{22} + c_{12}$	8.418	−1.071	−0.01047	0.5794	−7.654e−06	4.1

of bacteria growth during the bio-digestion is significant in determining the role of cultured SCAO in biogas production (Table 7).

On the other part, the relation of the biogas yield as against bacteria growth and time seems to be stable at different variations. Using the linear coefficients, quadratic coefficient, and cross-product coefficient in Table 5, the microbial growth was further monitored for declining growth scenario due to the lifespan of the SCAO in Fig. 19. Figure 19a, b, refers to the normal case in the experiment (Fig. 19a) and the test for declining growth (Fig. 19b) with respect to time. It was observed that though there was a variation of growth with respect to the biogas production in the laboratory experiment (Fig. 19a) when the growth of the bacteria is declining, the biogas production declines too (Fig. 19b). Secondly, there is the possibility that when the declined growth is at its lowest point, the biogas production is slightly stable at its lowest point. Figure 19c, d shows the influence of microorganism growth and biogas yield on the biogas production for normal or laboratory case (Fig. 19c) and the steady-growth case (Fig. 19d).

It was observed that when the growth of the microorganism is steady, the biogas yield and production are directly related to each other. In this case, the difference in biogas production between low and high bacteria populations is slightly significant. These theoretical estimations are mainly necessary for new regulations and legislation [48] to improve human excreta utilization via biogas production. From the above, the novel SCAO can be adopted to control ammonia and greenhouse gas emissions from human waste. This result means that there is still more to be done in culturing SCAO bacteria with a slow growth and longer life span to make this process appreciate to industrial scale.

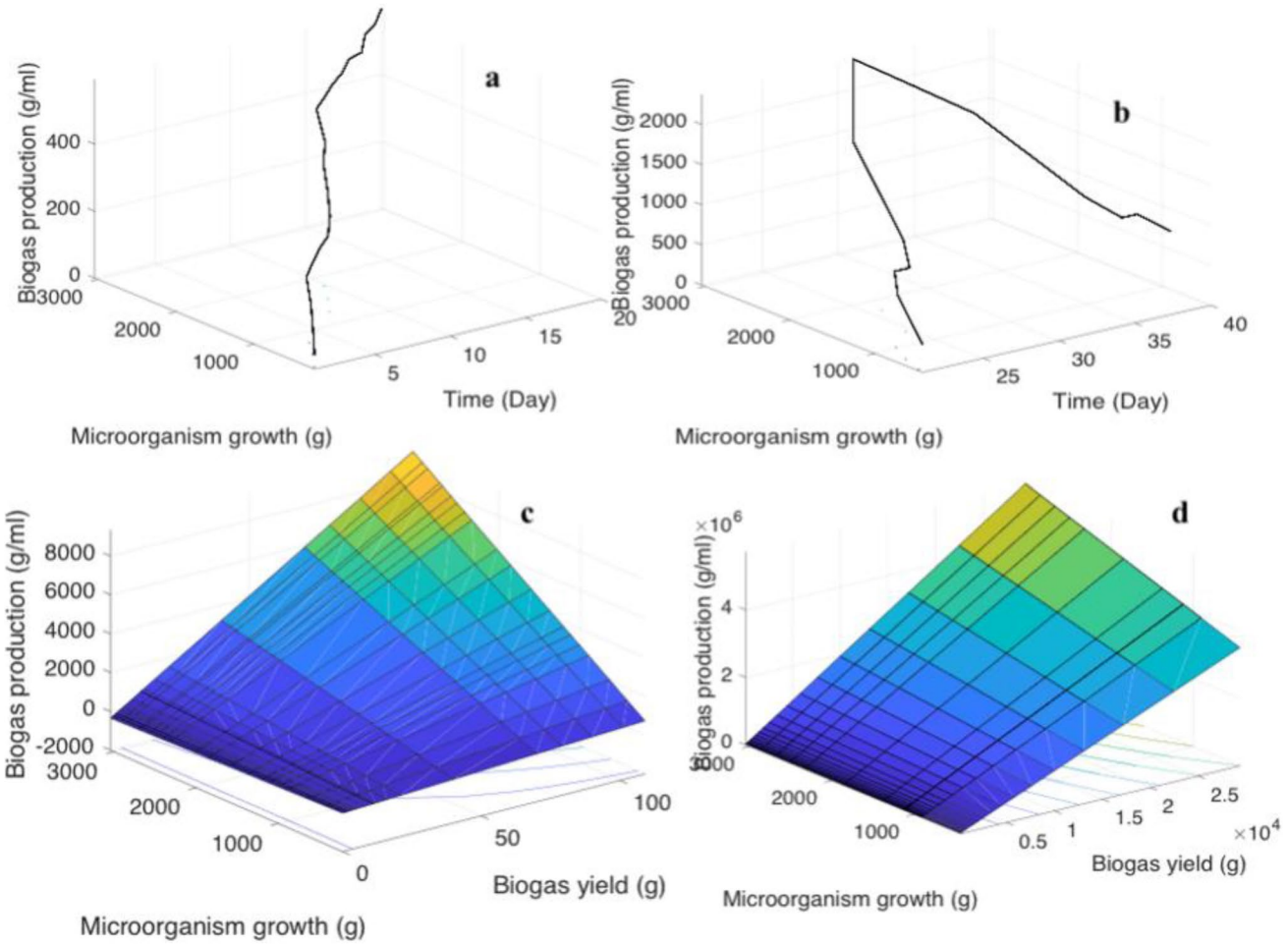

Fig. 19 Biogas production under microbial growth

7 Conclusion

At the end of this research, the SCAO bacteria was seen to produce significant biogas production that can be scaled up to the industrial application. It was clearly shown that aside from the design of bioreactor, biomass material, and digestion parameters, biogas production is dependent on the modified bacteria system. The novel SCAO bacteria are good candidates to control ammonia and greenhouse gas emissions from human waste.

The laboratory set-up used for this experiment could be scaled-up for home use. However, material selection becomes very necessary to avoid leakages or damage of any part of the bioreactor. It was reported that microbial growth plays a significant role as there were significant changes when the growth of the bacteria is declining, and when the growth is relatively stable. The SCAO bacteria were observed to decompose the ammonia gas to form methane and NOx gases at a ratio of 3:1 within the first 20 days. The results shows that this project can be scaled-up for commercial purpose of producing clean biogas.

This research has shown that impoverish homes in developing countries can afford this technology to solve 74% of its energy demands. This finding means that standalone users in developing countries could thrive with little or no legislative bottleneck. It is recommended that there is still more to be done in culturing SCAO bacteria with slow growth and longer life span to make this process appreciate to industrial scale.

Authors' contributions Emetere designed the research. All authors contributed to the write-up equally. The data curation was done by Chikwendu and Emetere. All authors read and approved the final manuscript.

Data availability Data shall be provided by corresponding author by written request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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