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Upgrading upflow anaerobic sludge blanket (UASB) reactors with rice straw biochar: a smart pathway for rural sanitation, bioenergy recovery& agricultural reuse

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Rural sanitation in Egypt faces critical challenges due to the high costs associated with conventional wastewater treatment systems. This study explores a low-cost, sustainable solution by integrating rice straw biochar, a locally available agricultural byproduct, into Upflow Anaerobic Sludge Blanket (UASB) reactors. Two pilot-scale reactors were operated at the Faculty of Veterinary Medicine, Suez Canal University: a standard UASB (R4) and a modified UASB (R3) amended with 2 g L^{-1} of rice straw biochar. Both systems treated buffalo cattle shed wastewater under identical conditions. The biochar-amended reactor (R3) significantly outperformed the conventional system, improving chemical oxygen demand (COD) removal from 79.9% to 86.0%, total suspended solids (TSS) removal from 74.0% to 81.6%, color removal from 72.7% to 81.8%, and turbidity from 75.7% to 81.9%. Biogas production also increased substantially, from 800 mL per day to 1500 mL per day, achieving a biogas yield of 0.050 L per g COD removed—an 80% improvement over the control. These enhancements are attributed to biochar's conductive and porous structure, which promotes microbial colonization and efficient electron transfer during anaerobic digestion. The study further demonstrates the agricultural reuse potential of the treated effluent, showing positive impacts on the growth of drought-tolerant plants and improvements in soil fertility. Rice straw biochar serves as a sustainable, locally sourced alternative to synthetic additives, aligning with circular economy principles. This integrated approach addresses sanitation, renewable energy production, and agricultural reuse, contributing directly to UN Sustainable Development Goals 6 (clean water), 7 (clean energy), 12 (responsible consumption), and 13 (climate action).

1 Introduction

Anaerobic digestion (AD) is a highly efficient and cost-effective process that employs specialized bacteria in several stages, such as hydrolysis, acidogenesis, acetogenesis, and methanogenesis. This process converts wastewater and organic waste into energy.[1] AD is affected by several factors, such as organic loading rate (OLR), hydraulic retention time (HRT), pH, and temperature.[2] Exceeding the ideal OLR can lead to a higher concentration of acidogenic bacteria than methanogens, while low HRT values can result in the washout of important microbial communities. AD's performance can be enhanced by providing support material for slow-growing microorganisms, thereby stabilizing microbial populations during high OLR and low HRT.[3]

The OLR is a crucial operational parameter that influences microbial activity, chemical reaction rates, and the biogas produced. The methane production is constrained by the degradation of fatty acids induced by obligatory syntrophic bacteria, which convert volatile fatty acids into carbon dioxide and acetate.[4] Methanogens utilize the electrons released from metabolic processes to transform carbon dioxide (CO_2) and acetate into methane (CH_4). Lower OLR values may lead to microbial malnutrition or the development of microorganisms with diminished activity. On the other hand, higher OLR can promote rapid proliferation of microorganisms, thereby enhancing the removal of organic matter and biogas production.[3] Sudden elevation of OLR can increase the rate of short-chain fatty acid production, leading to the buildup of intermediate products. This buildup can disrupt the stability of the

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community of microorganisms responsible for methane production (the methanogenic community).[5] Therefore, increasing the rate of symbiotic metabolism can potentially optimize methanogenesis.

Biochar has the potential to stabilize AD communities. Electrons involved in syntrophic metabolism can be transferred to methanogens *via* a direct or an indirect pathway. Indirect electron transfer often involves using hydrogen or formate as mediators to shuttle electrons between different species. Conductive materials such as granular activated carbon, carbon cloth, and biochar can facilitate direct electron transfer.[6] Biochar is a solid, granular substance produced by pyrolysis and carbonization in the absence of oxygen, transforming biomass into carbonaceous material.[7] Biochar possesses notable attributes, including high porosity, a significant specific surface area, exceptional stability, low bulk density, low electrical conductivity, and robust adsorption capacity. However, it also exhibits distinct properties and varying adsorption capabilities, depending on the biomass used as the raw ingredient.[8] Thus, while biochar is a cost-effective alternative to traditional activated carbon because it uses locally sourced waste biomass as its primary feedstock,[9] it is important to understand how different biochars will affect AD performance.

Biomass sources can affect biochar yield and porosity, which are usually higher in plant-based materials. Lignocellulosic biomass typically has a higher ash content than other sources, such as animal manure.[10]

Aya *et al.*[11] applied a robust and local method to produce biochar from harvested *Phragmites australis*, which were cut into small pieces and rinsed multiple times with clean water. The specimens were dried for 12 hours at 105 °C, then pulverized and screened through a 0.15 mm sieve. The biochar was produced by slow pyrolysis at 300 °C for 2 h with a heating rate of 10 °C min^{-1}. *Streptomyces hydrogenans* immobilized the produced biochar.

El Shahawy *et al.*[12] studied the effect of bioaugmented biochar, produced from common reed (*Phragmites australis*) with actinomycetes, on the efficacy of UASB. The investigation was conducted using a pilot-scale reactor. Adding biochar to the USAB reactor improved removal efficiency and biogas production.

In the current study, rice straw, an agricultural byproduct, is used to produce biochar. Egypt is considered the largest rice producer in the middle east, which is an important strategic crop. The total amount of agricultural crop residues contributing to biomass is about 12.33 million tons/year, of which 63.75% is rice straw. Rice straw causes serious environmental problems, as open field burning was, for decades, considered a means of rapid disposal. Open burning produces substantial amounts of air pollutants that contribute to severe deterioration of ambient air quality.[13]

The main aim is to enhance UASB performance and reuse an important agricultural waste, "Rice straw", which poses serious environmental issues. Rice straw biochar not only acts as a conductive material to enhance microbial activity but also represents a sustainable way to reuse agricultural waste for ecological benefits. The objective was to identify the effect of biochar produced from rice straw to improve the performance of an on-site agricultural AD. Two pilot-scale UASB reactors were used in the field to evaluate the impact of rice straw-based biochar on the effectiveness of organic waste removal and biogas production during AD of cattle shed wash water. The results were,[12] which tested the hypothesis that rice straw biochar could provide good results.

2 Materials & methods

2.1 Analytical methods

The biogas was measured using the water displacement method. The chemical oxygen demand (COD), pH, color, Turbidity, total suspended solids (TSS), and total dissolved solids (TDS) were determined according to the Standard Methods for the Examination of Water and Wastewater.[14] The interested reader is referred to ref. 12 for the details of the methods/instruments of analysis.

2.2 Buffalo wastewater characteristics

The veterinary farm of Suez Canal University has successfully built and employed experimental reactors (At 30° 37′32.9N latitude and 32° 16′05.3E longitude). In the current study, buffalo wastewater was the effluent from buffalo wash, with variable characteristics, as summarized in Table 1, which agrees with the analysis by El Shahawy *et al.*[12]

2.3 Production and characterization of biochar

Biochar was produced as previously described in ref. 11 and 15 The rice straw was cleaned and cut into 1 to 1.5 centimeter pieces. The cut pieces were dried in the oven for 4 hours at 110 ° C. After drying, the pieces were pyrolyzed at 400 °C for 1 hour, followed by a second stage at 700 °C for 1 hour. The pyrolysis system comprises an oil shower radiator, a temperature regulator (thermometer), a three-way connector, a warming Pyrex jar, biodiesel, and a cold-water condenser. Such an experimental test rig was constructed and used for pyrolysis at a wide range of temperatures,[16] the rice straw biochar was analyzed using an "Axion" X-ray fluorescence (XRF) spectrometer with a 1-kW wavelength dispersion. Alterations in the mineralogical

Table 1 The characteristics of influent buffalo wastewater[a]

Parameters	Max.	Min.	Mean values
pH	8.5	7.1	7.8 ± 0.5
TSS (mg L^{-1})	720	182	451 ± 93
TDS (mg L^{-1})	965	446	705 ± 136
COD, (mg L^{-1})	2000	550	1275 ± 156
TN, (mg L^{-1})	56.0	40.0	48 ± 7
TP, (mg L^{-1})	32.60	31.0	31.8 ± 0.7
Alkalinity (mg L^{-1})	350	317	334 ± 17
Color, PCU	361	64	212 ± 28
Turbidity, NTU	320	24	172 ± 17

[a] The average was calculated for 32 samples, which were collected on different days over 11 months. The ± is the standard deviation of 31 replicates.

composition were analyzed using the X-ray diffraction technique (XRD) with a Bruker Co. model D8.[11]

Furthermore, the structure of rice straw biochar was examined using a Scanning Electron Microscope (SEM) – Philips XL 30 with modern energy-dispersive spectroscopy (SEM-EDX), both before and after adsorption. The operating conditions included a low vacuum level and a voltage of 30 kilovolts. The Nova Touch LX2, a Brunner Emmett Teller (BET) analyzer manufactured by Quanta Chrome Company in the USA, was employed to measure the surface area, pore size, and volume both before and after adsorption. The measurements use the conventional volumetric technique involving nitrogen adsorption at 77 kelvin. The functional groups of rice straw biochar were detected using the PerkinElmer Model 1720 FTIR (Fourier transform infrared spectrometer). The spectra were acquired by scanning ranges from 40 to 4 m^{-1} with a resolution of 0.02 m^{-1}. The samples were generated by forming pellets of the product using 1 g of potassium bromide per 100 g of product.

2.4 Reactor setup and operation

The UASB reactor with a working volume of 5 liters was utilized. The reactors were fabricated from polyvinyl chloride (PVC) cylinders measuring 0.7 meters in height and 0.1 meters in diameter. The reaction zone was 0.65 meters from the bottom of the cylinders (Fig. 1a).

The system was operated at ambient temperature. The produced biogas was discharged by flowing through the pipe, ensuring that the settling zone remained undisturbed. After that, the wastewater was directed to the settling zone, where a small amount of suspended particles settled. Consequently, the particles were retained within the reactor while the biogas was efficiently separated using the three-phase separator. The collected effluent was obtained at the highest point of the reactor after the wastewater was consistently supplied to it through the bottom using a submerged pump. Using a submerged pump (JET HVT-750F 1 HP), sewage was conveyed from a ground storage tank to the reactors. To mitigate flow rate variability, a reservoir with a consistent water level was installed upstream of the reactors. A distribution device was affixed to the lower section of the column to ensure a steady supply of waste material to the reactor. A device for quantifying the amount of biogas generated was attached to the upper part of the reactor using a differential-pressure (DP) based flowmeter.[12]

Two cylindrical UASB bioreactors (conventional and modified UASB) were used. Both R4 (conventional UASB) and R3 (modified UASB) were fed with buffalo cattle shed wastewater without any additions starting on the first of September (Fig. 1b and c).

R3 (modified) was provided with rice straw biochar on the first of January with a concentration of 10 g L^{-1} (50 g) through an inclined inoculation pipe (1.5-inch diameter). The optimum dosage of biochar is 10–15 g L^{-1}.[17] The influent COD concentrations ranged from 550 to 2000 mg L^{-1}, and the HRT was 4 hours with an influent rate of 30 L per day. Effluent samples were collected and analyzed twice weekly at ambient temperature. No pH adjustment was made in the reactors, as it

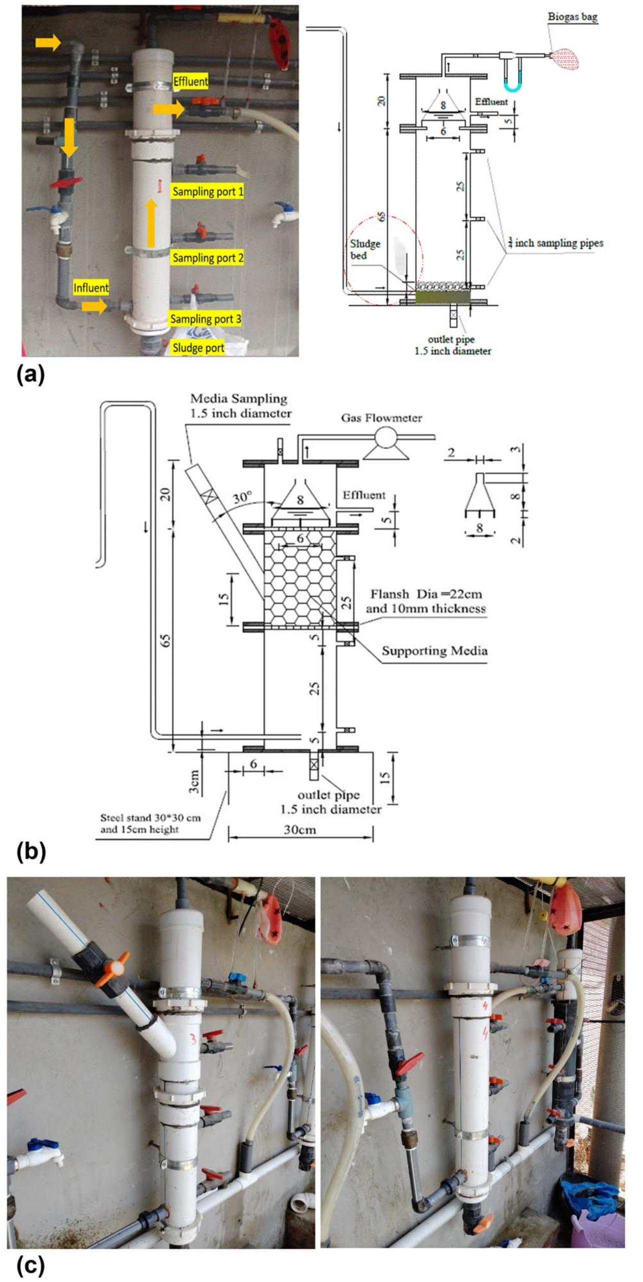

Fig. 1 (a) The schematic representation of the UASB reactor. (b) The schematic representation of the modified UASB reactor. (c) R4 (conventional UASB) and R3 (modified UASB).

remained consistently within the range of 7.2 to 8.5. Following the initiating reactor stabilization, rice straw biochar was introduced into the modified UASB reactor (R3) *via* an angled arm.

3 Results and discussion

3.1 Characterization of rice straw biochar

3.1.1 X-ray diffraction. Fig. 2a and b depict the XRD characterization of rice straw biochar before and after adsorption. The results showed that at COD 00-038-0448, a characteristic

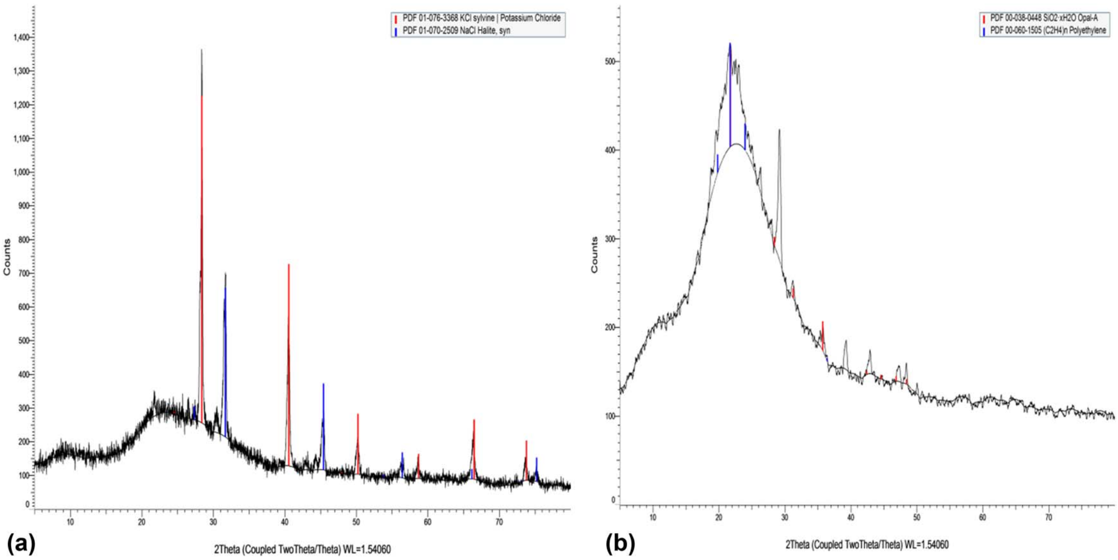

Fig. 2 (a) XRD of rice straw biochar before adsorption. (b) XRD of rice straw biochar after adsorption.

$SiO_2 \cdot xH_2O$ Silica (SiO_2) peak at 2 Theta positions ~28, 31, and 36. Also, the figure reveals the presence of COD 00-060-1505 $(C_2H_4)_n$ after adsorption, while, before adsorption, COD 01-076-3368, a potassium impurity as potassium chloride, was found at 2 theta 28, 41. It is well known that rice straw has a low lignin content and high Si and K levels. Thus, it is expected that the rice straw biochar will have a high content of SiO_2 and KCl.

Additionally, rice straw loses its crystallinity at 400 °C. Consequently, in the present work, the XRD pattern of rice straw biochar (RSB) indicates an amorphous, poorly crystalline, and carbon-rich material. After adsorption, the absence of SiO_2 indicates exchange with other metals and impurities in KCL.

3.1.2 Scanning electron microscopy (SEM). Fig. 3a and b show SEM images (at 3000× magnification) of rice straw biochar before and after adsorption. The SEM images show that, before adsorption, the RSB consists of irregular plates with a high surface area and porous structure. After adsorption, it shows a smooth surface, which indicates the removal of elements from wastewater.

3.1.3 FTIR spectroscopy. The removal of metal ions from wastewater depends strongly on the surface characteristics of rice straw biochar. The surface composition can be analyzed by FTIR spectroscopy. Fig. 4 shows the FTIR spectrum of rice straw biochar (RSB) before and after adsorption, respectively.

The figure shows several functional groups, including the OH group at 3430 cm^{-1}. After adsorption, the peak decreased to 1605 cm^{-1}, which shows C–C olefinic and C═O carbonyl groups. On the other hand, at 1430 cm^{-1}, the cyclic structure doesn't change. Finally, SiO_2 at 1104 cm^{-1} and aliphatic CH_2 at 618 cm^{-1} may be important for removing heavy metals from wastewater.

3.1.4 The brunner emmett teller analyzer (BET). The BET determines the particle size, pore volume, and surface area of solid porous materials. Fig. 5a and b display the BET of RSB

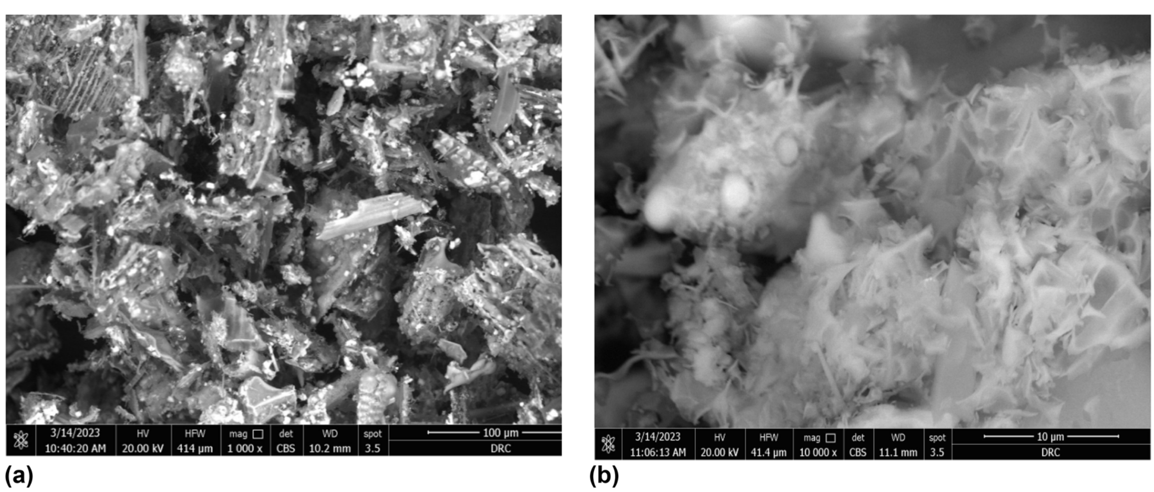

Fig. 3 (a) SEM RSBC before adsorption. (b) SEM RSBC after adsorption.

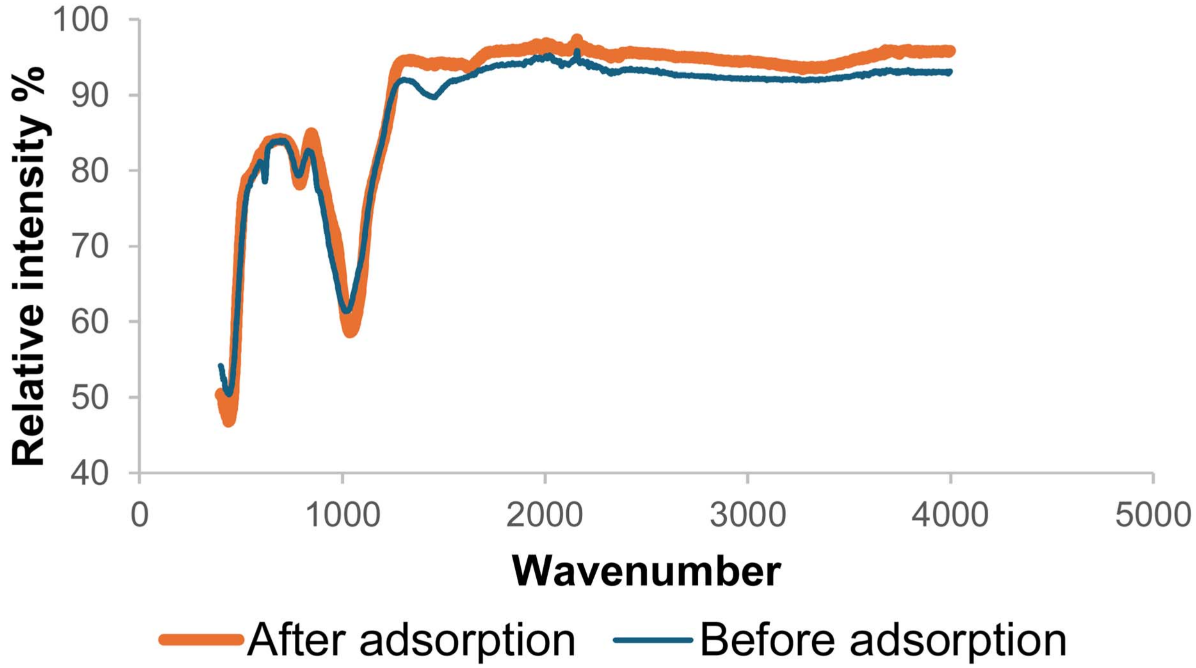

Fig. 4 FTIR diagram of rice straw biochar before and after adsorption.

before and after adsorption, respectively. The pore size and surface area were analyzed using the nitrogen adsorption–desorption analyzer. The specific surface area of RSB before adsorption was observed to be 348.25 m^2 g^{-1}, while the value after adsorption was 200.11 m^2 g^{-1}, as summarized in Table 2. This may be attributed to the additional surface area induced by the impurities in the biochar matrix. The pH value significantly affects an adsorbent's surface area, surface charge, and chemistry. Therefore, it is highly relevant to determine the point of zero charge (pH_{pzc}), the pH at which the adsorbent surface is electrically neutral. When the pH is below pH_{pzc}, the adsorbent has a net positive surface charge. If the pH is greater than the pH_{pzc}, the adsorbent surface will be negatively charged. The pH_{pzc} values of the adsorbents before and after adsorption of impurities from wastewater samples in this study were determined to be 7.8.[18–20]

3.1.5 XRF & EDX. Table 3 summarizes the XRF analysis of rice straw biochar after adsorption. It is clear from the table that Al_2O_3 and SiO_2 are the major constituents, while K_2O is present in trace amounts and is responsible for adsorbing organic matter, particularly organic load (COD). In XRF and EDX analyses, the adsorbent is highly efficient at removing metal ions from wastewater. Still, only rice straw biochar takes Na_2O and Br. It has lipped rice straw biochar with great efficiency uptakes for MgO, SiO_2, KCl, K_2O, CaO, and Br. Rice straw biochar has great efficiency and is a no-cost bioadsorbent. It shows great stability in metal ion removal at different pH values because it involves functional group interactions at the O–H group and the cyclic ring, as well as the exchange of Si metal with other ions, increasing the likelihood of removing ions from wastewater; see analysis in Fig. 6b. After adsorption, there is an increase in metal ions uptake from samples and a reduction of H^+ and O_2^- concentrations according to the breaking of the bond and formation of new bonds with metal ions. Fig. 6a displays EDX of rice straw biochar before adsorption.

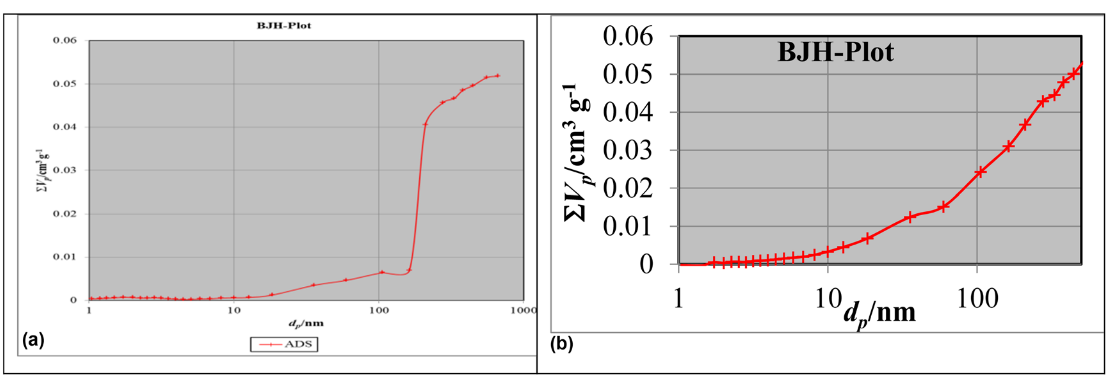

Fig. 5 (a) BET of rice straw biochar before adsorption. (b) BET of rice straw biochar after adsorption.

Table 2 Measured parameters of biochar before and after adsorption

	Before adsorption	After adsorption
Surface area S_{BET}, $m^2\ g^{-1}$ RSB	348.25	200.11
Pore volume, cm^3 per g RSB	0.033	0.065
Pore size, nm RSB	49.268	17.213

Table 3 XRF of adsorbents

Constituents wt% of rice straw biochar	
Na_2O	13.30
MgO	4.10
SiO_2	47.30
P_2O_5	0.911
Cl	4.59
K_2O	5.62
CaO	1.32
$Fe_2O_3^{tot.}$	0.227
SO_3	1.85
Zn	0.0176
Sr	0.0083
Br	0.0156
L. O. I	21.1

3.2 Wastewater treatment

3.2.1 COD removal. Fig. 7 displays the COD removal efficiency and the influent and effluent concentrations for the investigated UASB systems. The analysis indicated that the incoming wastewater COD concentration is 1018 mg L^{-1}, with minimum and maximum values of 550 mg L^{-1} and 1345 mg L^{-1}, respectively. In the conventional reactor, the effluent COD concentration reached 210 mg L^{-1} without inoculation, and the removal efficiency was 79%. In contrast, the reactor modified by introducing biochar achieved a COD of 140 mg L^{-1}, resulting in 86% removal efficiency. The results demonstrated no association between influent and effluent COD concentration fluctuations. More precisely, altering the COD in the influent wastewater did not lead to a proportional change in the effluent COD concentration. The HRT is responsible for the delayed response of the effluent COD concentrations to changes in the influent COD concentrations. Furthermore, it was observed that the effluent COD variations were less pronounced than the changes in influent COD concentrations.

The COD values were statistically analyzed using a paired-samples *t*-test in IBM SPSS statistics. The *t*-test revealed that the average effluent COD for the conventional UASB reactor was 250 mg L^{-1}, while it was 149 mg L^{-1} for the modified reactor. The *t*-test, using 31 degrees of freedom, yielded a *p*-value approaching zero (p-value = 0.001 < 0.05), significantly lower than the 5% significance level. The lower computed *p*-value confirmed that the modified UASB system improved considerably compared to the traditional system.

El Shahawy *et al.*[12] investigated the effect of bio-augmented biochar with actinomycetes from *Phragmites australis* on the UASB efficiency. They concluded that the bioaugmented biochar with actinomycetes system achieved 93% COD removal, compared with 81% for the conventional system.[12]

A similar conclusion was reported in previous work using activated carbon,[19] in which a traditional UASB and a granular activated carbon (GAC)-amended UASB were used to treat municipal wastewater for 225 days. The treatment was conducted at different hydraulic residence times ranging from 36 to 8 hours. The COD removal efficiencies ranged from 69 to 83% for the UASB reactor with granular activated carbon amendment and from 58 to 66% for the UASB reactor without GAC. The findings[21] from the laboratory-scale hybrid UASB reactor indicated that the HRT ranged from 6 to 24 hours, with an organic load fluctuating between 4.8 and 20.99 kgCOD m^3 per day. The COD removal efficiency reached 85.57% on the 171th day, with a chemical oxygen demand loading rate of 5.2 kgCOD m^3 per day and an HRT of 24 hours.

Utilizing biochar as a carrier material facilitates synergistic interactions with microbes through its multifaceted functions, including nutrient provision, colonization promotion, establishment of a suitable environment for microorganisms to thrive, and effective removal of harmful organic pollutants from wastewater.[22]

The findings suggest that hydrolysis occurs initially, followed by the formation of the acid substrate, and subsequently, the methanogenic substrate is present in the reactor after acidification. It explains the initial period of falling COD

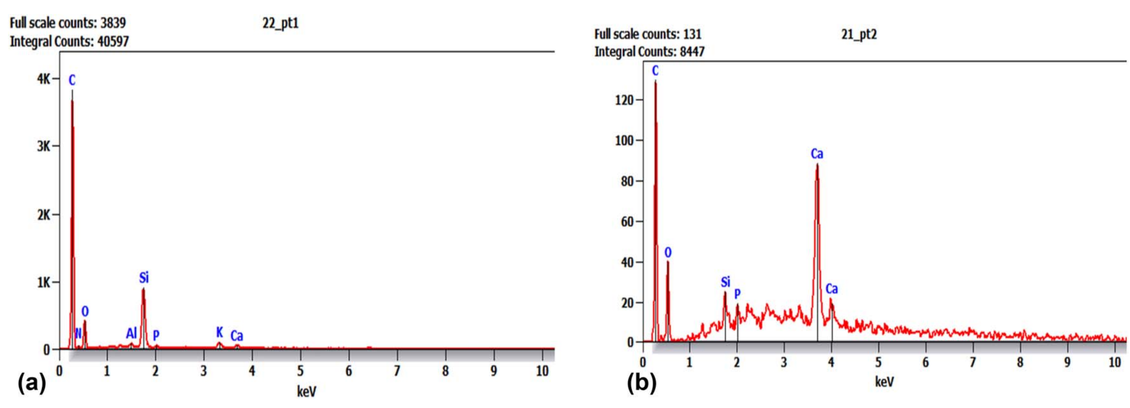

Fig. 6 (a) EDX of rice straw biochar before adsorption. (b) EDX of rice straw biochar after adsorption.

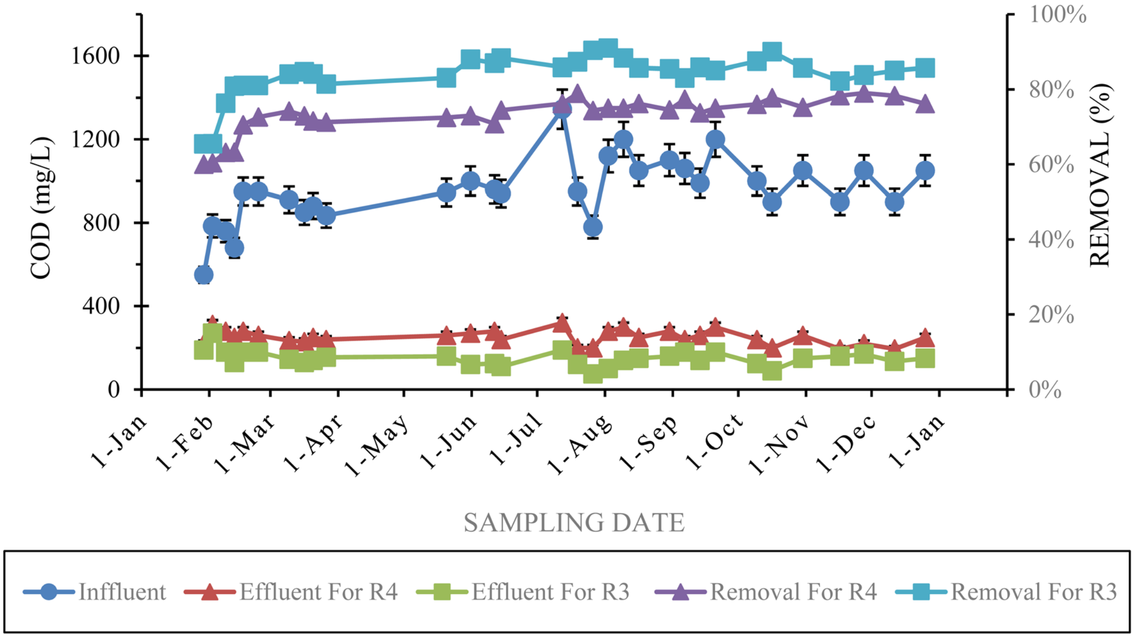

Fig. 7 COD removal from UASB systems. R4: traditional UASB, R3: modified UASB with biochar.

removal efficiency. Insufficient conversion of the solubilized products occurs when the populations of acidogenic, acetogenic, and methanogenic organisms are negligible, resulting in their accumulation in the effluent.[23]

3.2.2 Suspended solids removal. Fig. 8 displays the effectiveness of TSS removal and the inlet and outlet concentrations in the operated UASB systems. The inlet TSS concentration averaged from 530 mg L^{-1} to 182 mg L^{-1}, with a mean value of 412 mg L^{-1}. In the standard conventional reactor, without the addition of any ligand, the effluent exhibited a peak of 150 mg L^{-1} and a minimum of 70 mg L^{-1}. Conversely, in the inoculation reactor, the lowest TSS was 50 mg L^{-1}, while the highest was 120 mg L^{-1}. Additionally, it was observed that TSS levels in the incoming wastewater were lower during the three weeks of influent than in the subsequent weeks. This could be due to the incomplete establishment of microbial communities during the opening weeks of UASB reactor operation. The system is now undergoing microbial maturation, and the effectiveness of TSS removal may improve as the microbial population stabilizes and becomes more active.

SS clearance efficiency was inferior throughout the early period in the absence of inoculation compared to the presence of inoculation. This may be attributed to the first run of the empty (traditional) reactor, during which the sludge quantity was minimal. The initial period is a crucial stage in which microorganisms are developed and adjusted to the specific properties of wastewater. The TSS removal efficiency reached 74% in traditional reactors. Rice straw biochar facilitates the decomposition of organic matter, thereby enhancing TSS removal under inoculation. In addition, rice straw biochar acts as an adsorbent, effectively trapping suspended matter and additional contaminants in the wastewater. The TSS removal efficiency reached 80.6% in the modified system.

The TSS measurements are subjected to a paired *t*-test. The average TSS outlet concentration in the conventional reactor was 116 mg L^{-1}, whereas it was 79 mg L^{-1} in the modified system. The *t*-test used 31 degrees of freedom and yielded a *p*-value approaching zero (p-value $= 0.001 < 0.05$), which is lower than the 5% significance level. The lower computed *p*-value indicates a considerable drop in outlet TSS readings of the improved UASB compared to the values of the conventional system.

Similar results were obtained when a UASB system was applied to treat raw sewage at Pedregal. The TSS elimination efficiency was precisely quantified at 63% when the HRT was set at 17 hours.[23,24] The results were reported;[25] the study examined wastewater processing in the dairy sector using an UASB followed by an activated sludge reactor. The HRT of the UASB was 24 hours, with average OLRs of 3.2 kg COD per m^3 per day. The average TSS clearance exceeded 72%.

The results were reported[26] by applying the UASB technique to treat wastewater generated by a mechanical shucking and clam-processing operation. The UASB system demonstrated its effectiveness by achieving a TSS removal rate of 83% under optimal conditions, with an average OLR of 13.8 g COD per L per day and an average HRT of 3 hours. The results were obtained[27] using a UASB reactor to process olive mill effluent. The UASB system was operated during 477 days at OLRs varied from 0.45 to 32 kg COD per m^3 per day, and the TSS removal efficiency was 64.6%.

3.2.3 Dissolved solids removal. Fig. 9 shows the TDS removal efficiency and inlet and outlet concentrations for the studied UASB system. The diagram shows that the mean TDS in the incoming flow was 717 mg L^{-1}, with the highest and lowest recorded values of 1204 mg L^{-1} and 590 mg L^{-1}, respectively. In the traditional system, the effluent reached its highest TDS concentration of 470 mg L^{-1} and its lowest of 160 mg L^{-1}.

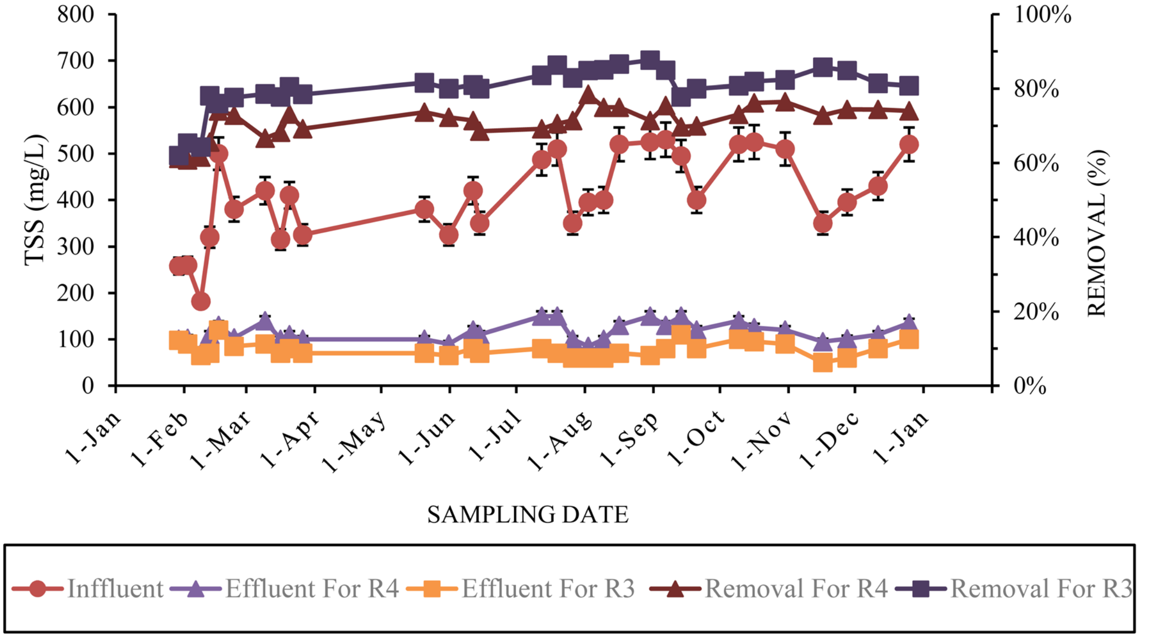

Fig. 8 TSS removal from UASB systems. R4: traditional UASB, R3: modified UASB with biochar.

Conversely, in the modified reactor (with inoculation), the lowest TDS concentration reached was 90 mg L^{-1}, while the highest concentration was 334 mg L^{-1}. Regarding the concentration of TDS in effluent, it was observed that pollutant levels in the effluent varied, similar to those in the influent, throughout the operation. Furthermore, there was a disparity in the effluent TDS concentrations between the two cases, indicating that introducing microorganisms affected the elimination or alteration of dissolved solids in the wastewater.

In the case without inoculation, the early period showed reduced TDS clearance effectiveness compared to when inoculation was implemented. In the absence of inoculation, the TDS removal efficiency reached 69.62%. Regarding inoculation, the TDS elimination rate reached 80.37%.

The results of the present study agreed with those of other researchers; the UASB reactors used to treat household wastewater had TDS removal efficiency ranging from 65 to 85%.[28] Similarly, the UASB process achieved removal efficiencies of 83–

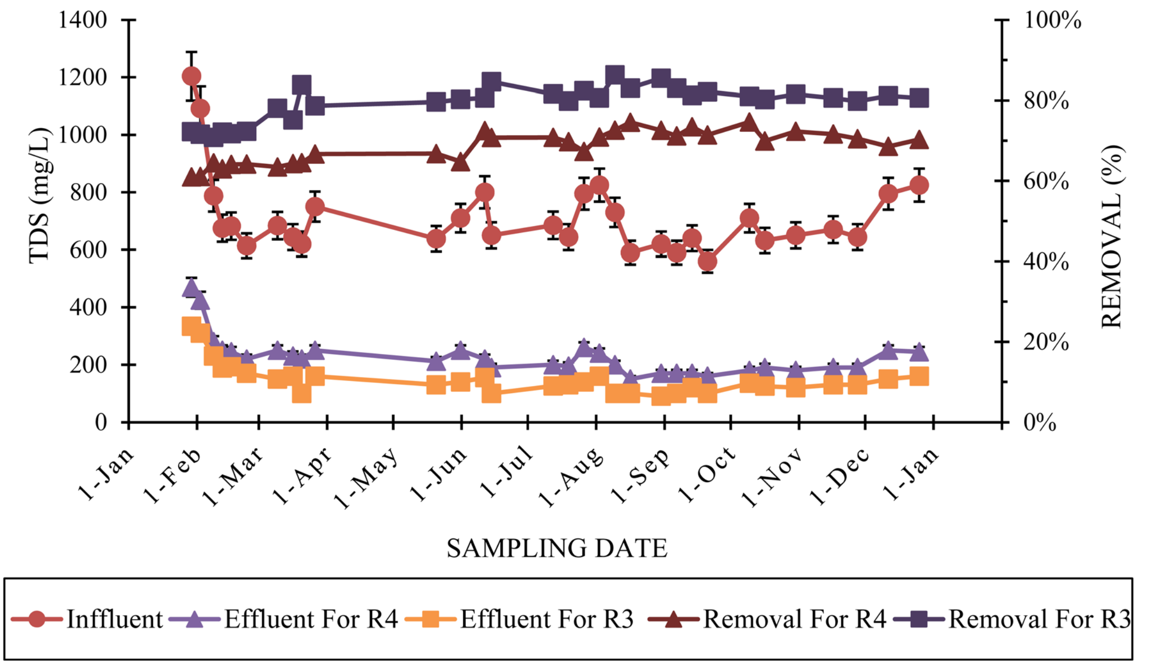

Fig. 9 TDS removal in UASB systems. R4: traditional UASB, R3: modified UASB with biochar.

85% for total dissolved solids in both fortified municipal wastewater and synthetic sewage with high strength.[29,30] The results were obtained.[31] The two identical UASB reactors, one inoculated with cow sludge (UASBCS) and the other with activated sludge from a dairy wastewater treatment plant (UASBASDIT), were brought back into operation after being idle for 12 months. The TDS removal in the operated reactors exhibited substantial fluctuations (4–22%) during the first 28–30 days, but reached a stable state over the following 65 days.

3.2.4 Color removal. Fig. 10 shows the color removal efficiency and the inlet and outlet color concentrations. The inlet color concentration was 181 PCU (Platinum Color Units), with an average value, as seen in the figure, with a highest value of 235 PCU and a lowest value of 125 PCU. In the conventional reactor, without the addition of any external substances, the most intense color concentration at the outlet was 100 PCU, while the lowest was 50 PCU. Conversely, in the improved inoculated system, the lowest color concentration reached 33 PCU, while the highest was 75 PCU. The color removal efficiency at the traditional reactor, without inoculation, was 72.72%. Regarding inoculation, the rate of color elimination has achieved 81.81%.

Rice straw biochar has adsorption capabilities that can impact the elimination of color. The biochar effectively adsorbs colorants from water, thereby enhancing the efficiency of color removal.[32]

Similar results were reported by other researchers,[33] who used two types of reactors—UASB and modified UASB—to treat cattle slaughterhouse wastewater. The reactors were operated at a 24-hour HRT, with a dosing pump operating six times daily. A water jacket heated the two reactors, and the operating temperature was maintained at 35 ± 1 °C. Decolorization was higher in the modified reactor even when loading rates were increased.

Similar results were obtained.[34] The UASB reactor treats simulated textile wastewater with a solitary dye, achieving a color removal efficiency of 71.0%. A modified configuration increased the HRT to 48 hours, maintained an outlet color concentration of 50 mg L^{-1}, and achieved a color removal rate of 77.8%.

3.2.5 Turbidity reduction. Fig. 11 depicts the turbidity removal efficiency and the inlet and outlet concentrations. The average influent turbidity concentration was 104 NTU (Nephelometric Turbidity Unit), as shown in the figure, with a maximum of 150 NTU and a minimum of 80 NTU. In the traditional reactor, the highest outlet turbidity concentration was 60 NTU, while the lowest was 25 NTU. In contrast, the modified reactor, which was inoculated, had a minimum turbidity concentration of 18 NTU, while the maximum Turbidity was 50 NTU. In the conventional reactor, the removal efficiency was 75.70%, whereas in the inoculated reactor, it reached 81.92%.

The Turbidity measurements were subjected to statistical analysis using a paired *t*-test. The average Turbidity at the outlet of the conventional reactor was 38.5 NTU, while the modified, inoculated reactor had an average of 29.9 NTU. The *t*-test used 31 degrees of freedom and yielded a *p*-value of 0.001, which is significantly lower than the 5% significance level. The lower computed value indicates a considerable drop in the inoculated UASB outlet concentrations compared with the conventional system.

Similar results for removing Turbidity were reported in previous studies[35] using a lab-scale UASB operated over 116 days. Artificial municipal effluent was utilized as the source material. The average outlet turbidity was 55 NTU with a water upflow velocity of 1.2 m h^{-1}. However, it fell to 32 NTU when the water velocity was reduced to 0.6 m h^{-1}.

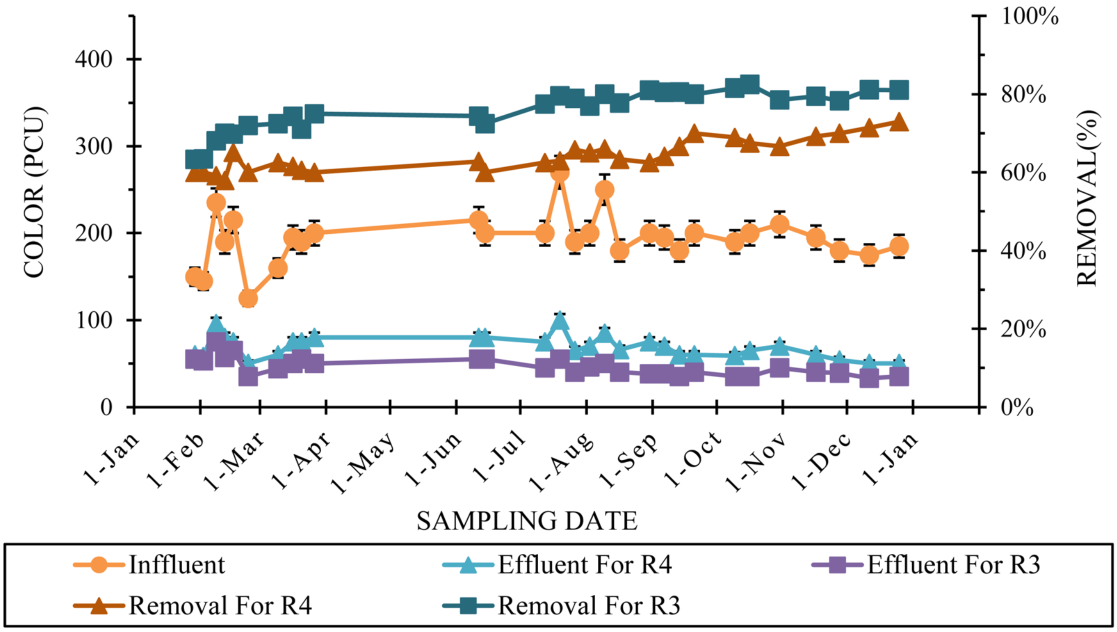

Fig. 10 Color removal in UASB reactors. R4: traditional UASB, R3: modified UASB with biochar. PCU, Platinum Color Units.

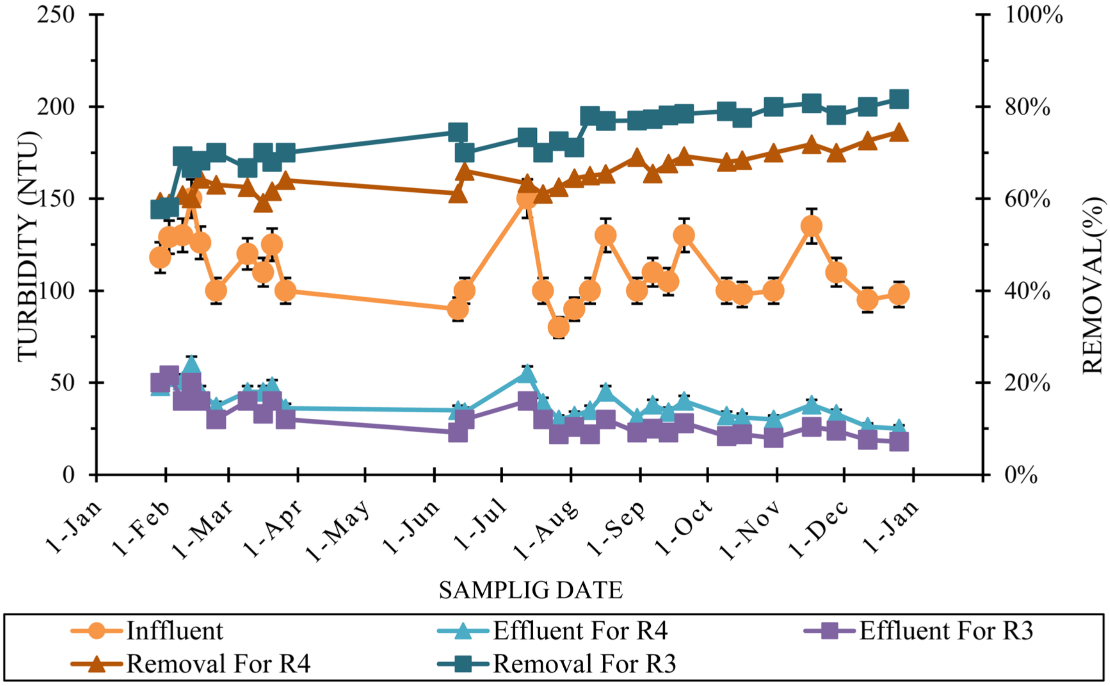

Fig. 11 Turbidity measurements for UASB reactors. R4: traditional UASB, R3: modified UASB with biochar. NTU, Nephelometric Turbidity Unit.

Similar results were obtained.[36] This study employed two types of reactors—conventional (R1) and modified UASB (R2)—to treat cattle slaughterhouse wastewater. The HRT of the reactors is retained for 24 hours. Turbidity is a crucial characteristic in assessing wastewater treatment system performance. The main conclusion was that the turbidity removal depended on the loading rates. At organic loading rates of 1.75, 3, and 5 g per L per day, the removal efficiency was 92%, 92%, and 75% in R1, and 96%, 94%, and 90% in R2. However, when the OLR was increased to 10 g per L per day, turbidity removal dropped to 41% for R1 and 81% for R2.

3.3 Biogas production

A differential-pressure-based flowmeter with a U-tube design is the primary technology for monitoring biogas flow. This flowmeter operates by measuring the decrease in pressure across a constriction in the direction of the flow (as shown in Fig. 12).

The total biogas flow rate was determined by measuring the pressure differential, and the biogas was then collected in a gas bag. The biogas production was increased during operation of the two systems until the reactors reached near steady-state. Fig. 13 shows that the biogas production rate was higher in the inoculated modified reactor than in the conventional reactor. In the conventional system, the maximum biogas production was 800 ml per day, whereas it was 1500 ml per day in the inoculated system.

The biogas production data were analyzed using a paired *t*-test. The results indicated that the average biogas production volume for the original system was 660 ml per day, but it was 1214 ml per day for the upgraded system. The *t*-test used 31 degrees of freedom and yielded a *p*-value of 0.001, which is significantly lower than the 5% significance level. The lower computed *p*-value indicates a significant increase in biogas output from the inoculated system compared to the conventional system.

The conventional reactor (R4) has a C/N ratio of 14. Eventually, it further increased to 800 mL per day after ten months of continuous operation. Initially, the pH increased from 6.5 to 7.11, and thereafter continued to rise to a range of 7.11 to 8.1 after 10 months. The pH increase affected microbial activity in the reactor. Nevertheless, anaerobic digestion operates within a specific pH range, typically 6.8 to 7.5. The pH range varies based on the substrate and the specific digesting technique implemented.[37]

Conversely, the modified reactor (R3), which had a C/N ratio of 17, exhibited more substantial enhancements in biogas production rate throughout ten months of uninterrupted operation. The findings of this study demonstrate substantial fatty acid degradation and effective anaerobic digestion of the organic material, particularly in the reactor with the highest carbon-to-nitrogen ratio.

Most of the biogas production was attributable to inoculation. It tended to increase in tandem with COD removal efficiency; the COD removal ratio reached 86% when the equivalent cumulative biogas production was 1500 mL per day. The gas production rate increases as the COD value increases to the optimum level. This is due to the higher abundance of organic chemicals available for bacteria to convert into gaseous substances.

The obtained outcomes demonstrate substantial fatty acid degradation and effective anaerobic digestion of the organic material, particularly in the reactor with the highest carbon-to-

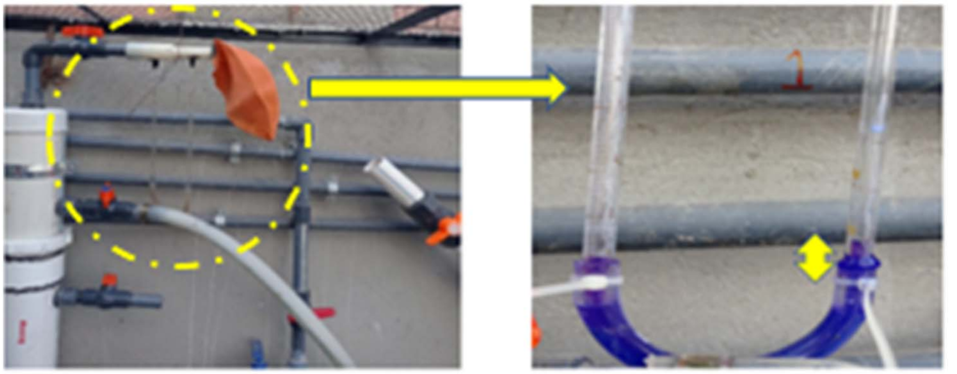

Fig. 12 A differential pressure-based flowmeter with a U-tube.

nitrogen ratio. Another study[38] assessed the effectiveness of two laboratory-scale UASBs, R1 (standard) and R2, which are modified to treat cow slaughterhouse sewage under mesophilic conditions. The R2 reactor achieved a noteworthy COD elimination efficiency of 94% during steady-state operation. Additionally, biogas production amounted to 2700 mL, with a methane content of 89%. Furthermore, a UASB system was assessed by[39] for treating slaughterhouse wastewater. The reactor produced 11 liters of biogas per day at standard temperature and pressure. The COD removal ratio was 77% when the average organic loading rate was 6.5 (kg COD m^3 per day).

The enhanced reactor performance and biogas production could also be attributed to the addition of biochar. The porous structure of biochar provides space for microorganisms to thrive and form colonies and can also hold nutrients on its large surface area to support them, which can enhance volatile fatty acids (VFAs) degradation and increase the CH_4 production rate (23.0–41.6%). Furthermore, biochar enhances the stability of the anaerobic digestion process by adsorbing major inhibitory compounds and elements, such as heavy metals, toxins, and antibiotics. The presence of rich functional groups, aromatic groups, and amine groups makes biochar effective at adsorbing toxins while simultaneously hastening the degradation of VFAs.[10] The addition of biochar also accelerates the Direct Interspecies Electron Transfer (DIET) efficiency, thereby enhancing anaerobic digestion efficiency. The methane production rate increased from 4.0 mL per day to 10.4 mL per day with the addition of biochar. Improving DIET efficiency is mainly related to biochar's properties. The porous structure and rough surface morphology provide ample attachment sites for microorganisms, strengthen their attachment, and indirectly promote DIET.[17]

4 Discussion

There is limited attention given to the investigation of the treatment of different types of wastewater using UASB reactors,

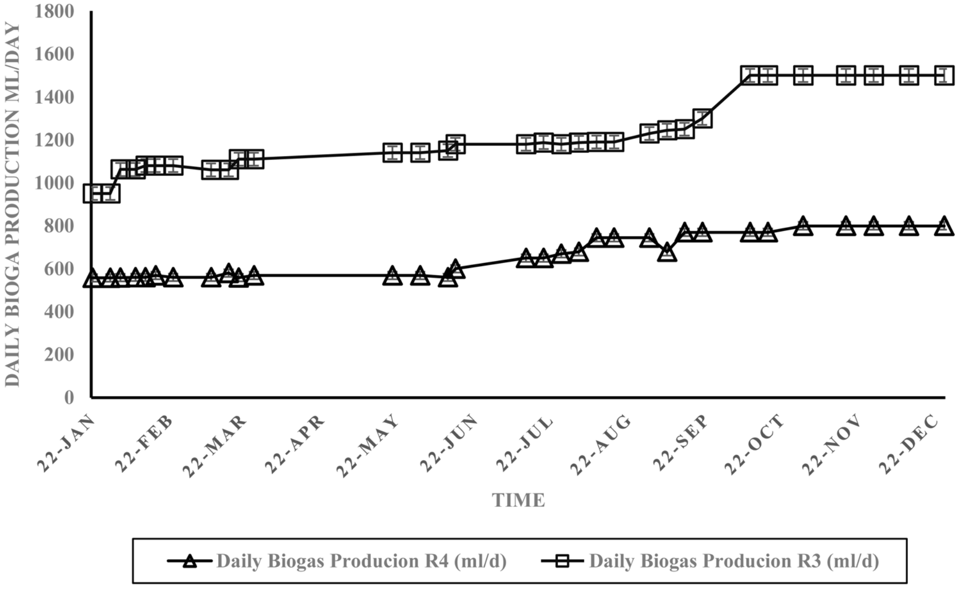

Fig. 13 Biogas production in UASB systems. R4: traditional UASB, R3: modified UASB with biochar.

Table 4 Comparison between volumetric biogas production with other researchers

No	The utilized reactor vol. (L)	Inlet COD (mg L^{-1})	Removal ratio (COD% %)	Volume of biogas produced (ml)	Carbone to nitrogen ratio	(ml biogas per mg COD removed)	Type of wastewater	References
1	5	1192	86	1500	17	0.050	Buffalo	Present study inoculated R3
2	5	1192	79	800	14	0.028	Buffalo	Present study conventional R4
3	24	18 000–20 000	92.5	328	49.5	0.001	Cassava	40
4	6	1300	90	3000	15	0.028	Dairy	41
5	0.8	10 000	95	220	20.1	0.029	Wheat straw	42
6	32.4	6250	80	2470–2980	21	0.002	Municipal	43
7	0.255	150 000	76.8	1453	—	0.049	Wheat straw	44
8	2750	866	66.5	427 000	—	0.270	Urban	45
9	10.2	20 000–30 000	80	20 000	25	0.30	Grain distillation	46

Table 5 Methane production average for the operating period

Reactor	C:N	Biogas efficiency ml biogas per mg COD removed	CH_4%	pH
Conventional R_4	14	0.028	73	7.9
Modified R_3	17	0.050	84	7.5

as the majority of research focuses on reactor construction principles and dimensional parameters. Physical characteristics, COD biodegradation, and biogas production from this study were compared to previously reported values (Table 4). In the current study, a direct side-by-side comparison between a traditional and a modified UASB showed that the modified UASB produced more biogas. The traditional UASB reactor (R4) achieved a biogas-to-COD removal ratio of 0.028 ml mg^{-1}, which was higher than the 0.020 ml biogas per mg COD reported in ref. 40 and 42, where the influent wastewater source was similar to that of R3 and R4. The ratio for the modified UASB (R3) was 0.05. Still, biogas volume alone is insufficient to understand a UASB reactor's efficiency, and methane production should be reported directly. Table 5 compares biogas production with the percentage of methane in the biogas. This comparison shows that the modified (R3) reactor was more efficient at recovering bioenergy from buffalo wastewater.

5 UASB effluent in agricultural reuse: a pathway to sustainable farming

A sustainable approach to reusing buffalo milkmaid wastewater for cultivating drought-resistant fodder shrubs. Using a pilot-scale UASB system, the study treated wastewater *via* sedimentation and anaerobic digestion (with and without rice straw biochar). Results showed that the modified UASB (R3) and conventional UASB (R4) achieved the highest COD and BOD removal (up to 93% & 81% COD removal, and 91% & 75% BOD reductions, respectively). Statistical analyses (split-plot ANOVA, correlation, PCA, and cluster analysis) confirmed that UASB-based treatments, especially R3, optimized vegetative growth and biomass while enhancing soil fertility. A bibliometric analysis of global wastewater reuse research (1999–2024) identified increasing interest in biochar-enhanced UASB systems and sustainable irrigation practices, with emerging opportunities to optimize biochar properties and integrate treatment technologies. The study concludes that treated buffalo milkmaid wastewater, particularly *via* modified UASB, offers a viable fertigation strategy for arid and semi-arid regions, contributing to sustainable water management, soil enrichment, and fodder production.[47]

The integration of UASB-treated effluent into agricultural practices holds significant promise for enhancing both water and soil sustainability. The UASB system, which efficiently treats wastewater, produces an effluent rich in nutrients such as nitrogen, phosphorus, and potassium—key components essential for plant growth. This treated effluent is increasingly being explored for agricultural reuse, particularly in water-scarce regions. Previous studies, including those investigating the use of UASB effluent from buffalo milkmaid wastewater, demonstrate its potential for irrigating drought-resistant species such as *Acacia saligna* and *Moringa oleifera*. By leveraging the nutrient-rich nature of UASB effluent, these plants benefit from improved soil conditions, leading to enhanced vegetative growth and biomass production. This agricultural reuse approach not only supports sustainable farming practices but also minimizes freshwater use and mitigates the environmental impact of untreated wastewater. The results from UASB-based treatments show substantial improvements in soil organic matter, nitrogen, and potassium content, ultimately contributing to better soil fertility and healthier crops. As global water scarcity increases, UASB effluent provides a viable solution to enhance agricultural productivity while maintaining environmental sustainability.

6 Conclusions

The main aim of our study was to investigate the utilization of the UASB reactor for buffalo wastewater treatment. The experimental work compared two pilot-scale reactors using high loadings of buffalo cattle shed wastewater: a traditional UASB and a UASB modified with biochar produced from agricultural waste (Rice straw). Adding biochar to the modified (USAB) reactor enhanced COD removal efficiency. The fieldwork pilot study approach also demonstrated that a UASB reactor could be started without seed sludge and maintain consistent stability even when challenged by variations in influent characteristics across multiple seasons. The use of UASB effluent for irrigation not only enhances plant growth and biomass production but also offers a sustainable approach to wastewater reuse, contributing to both agricultural productivity and environmental conservation.

Author contributions

N. N., S. S., H. L. G., A. E., D. A., A. D., and A. S. devised the research idea and initiated the study. A. D., D. A., A. E., and A. S. designed the experimental test rig and prepared a review background. N. N., S. S., D. A., and A. E. collected the materials and drafted the manuscript, which was critically commented on by H. L. G., S. S., D. A., A. E., A. D., and A. S. all the authors reviewed the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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