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Study of Biological Treatment of Rice Mill Wastewater Using Anaerobic Semicontinuous Reactors (ASCR)

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ABSTRACT

Anaerobic digestion (AD) of industrial wastewater has drawn researchers' attention due to biofuel's recovery in the form of biomethane. This study introduced two anaerobic semi-continuous reactors (ASCR)- R1 and R2 for bioremediation of the rice mill wastewater (RMWW). The alkali treatment of the substrate in reactors R1 and R2 was done by dry NaOH and $Ca(OH)_2$, respectively. Both reactors were loaded with 80% of the RMWW and 20% of the cow-dung-fed biogas plant sludge (BGPS) for 16 days of stabilization at mesophilic temperatures (18℃ to 42℃). A small amount of jaggery and white rot fungi (*Phanerochaete chrysosporium*) were also added into both reactors for the bacterial growth and removal of the biorefractory organics (lignin and phenol) present in RMWW, respectively. The impact of variations in the hydraulic retention time (HRT) and organic loading rate (OLR) upon the anaerobic biodegradation of RMWW was studied in three operating phases (OP) I, II, and III. The highest BOD, COD, lignin, and phenol removal achieved in reactors R1 and R2 were 94%, 92%, 84%, and 82%, as well as 93%, 91%, 82%, and 80%, respectively, in OP I. The highest biomethane yield in both reactors was 0.005 $L.g^{-1}$ COD in OP II. The results of the three operating phases reveal that a high HRT and low OLR give the maximum pollutant removal efficiency and the highest biomethane yield. The novelty of this research paper is the significant removal of the biorefractory organics lignin and phenol from the RMWW with the help of white rot fungi and specific bacterial strains *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Actinomycetes* sp. and *Streptomycetes* sp. present in the inoculum. The digestates from reactors were rich in macro and micronutrients viz., N, P, K, Cu, Zn, Fe, etc., essential for plant growth.

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INTRODUCTION

The rapid growth of the population, accompanied by economic progress through industrialization and urbanization, has resulted in sustainability challenges and climate change. The accelerating consumption of resources has resulted in the total waste generation of approximately 12 x 10^3 million tons (MT) due to anthropogenic activities in the year 2020, which is anticipated to be enhanced to 19 x 10^3 million tons per year ($MT.yr^{-1}$.) by 2025 (Duan et al. 2021). As per the Central Pollution Control Board (CPCB) report, nearly 72,368 million liters per day (MLD) of wastewater was produced by Class 1 cities and Class 2 towns in India in 2022. This wastewater consists of municipal and industrial wastewater, of which only 28% is remediated, and there is a broad gap of 72% of the untreated wastewater (Bassi et al. 2022). The huge amount of waste generated by various industries has led to an undeniable confrontation between industrial development and ecological sustainability. Industrial effluents have harmful effects on biodiversity because of their ambulant character. They are generally discharged into surface water bodies without appropriate treatment, creating consequential adverse damage (Alderson et al. 2015). Environmental sanitation is essential for the sound health of the citizens of a nation.

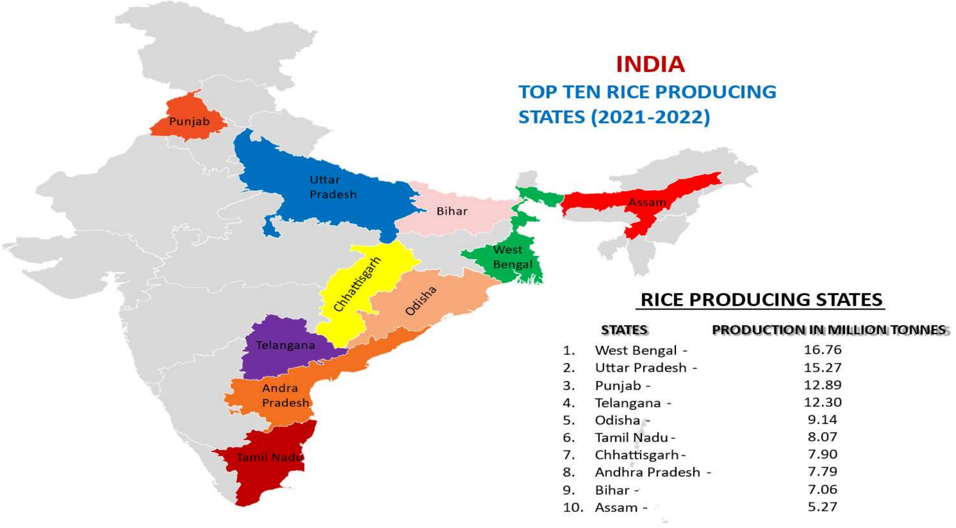


Fig. 1: Map of India showing the top ten rice-producing states in the financial year 2021-2022.

In the current era, agro-industries have gained momentum in size to fulfill people's food demands with a quickly growing population. Rice production from paddy milling is a progressively rising agro-industry and plays a pivotal role in the global circular economy. Rice is a staple food for a larger global population (Veluchamy & Kalamdhad 2017). The global rice production in the financial year (FY) 2021-22 was 513.06 MT. India produced 130.29 MT of rice in FY 2021-22, after China, which produced 148.99 MT in FY 2021-22. India's contribution to global rice production was 25.39%, whereas China's was 29.04% in FY 2021-22. Thus, India and China collectively contributed to more than half (54.43%) of the world's total rice production in FY 2021-22 (Paddy Outlook- March 2023). Asian countries contribute more than 90% of global rice production (Yusrin et al. 2024). India is the world's second-largest producer of rice and the largest consumer and exporter of rice. It stands first in the world regarding total acreage under rice cultivation. Besides fulfilling the domestic rice needs, India exports rice (basmati and non-basmati) to 150 countries worldwide (Annamalai & Johnson 2023). Fig. 1 shows a map of India showing the top ten rice-producing states in the financial year 2021-2022.

There are approximately 1,30,000 rice mills in India including Chhattisgarh, to date. The rice mills operating in India generally have two types- raw/white rice mills and parboiled rice mills, producing raw/white and parboiled rice, respectively (Singh & Bajpai 2023). The rice mills, producing parboiled rice discharge an enormous amount of wastewater in paddy soaking during the paddy parboiling process. Varshney (2012) investigated that one parboiled rice mill discharges 20 x 10^6 liters of wastewater per year in India. Thus, 57850 rice mills producing parboiled rice (COINDS 2008) release approximately 11.57×10^9 $L.yr^{-1}$. of wastewater in India.

There are two methods for rice mill wastewater (RMWW) treatment- physicochemical and biological. The physicochemical methods are cost-intensive and produce huge quantities of sludge, whose safe disposal becomes a big problem. On the contrary, the biological methods generate less sludge and are cost-effective (Singh & Bajpai 2023). Biological methods are capable of recovering green energy in the form of biomethane, biohydrogen, and bioelectricity with the help of anaerobic digestion (AD), dark fermentation (DF), and microbial fuel cells (MFCs) techniques, respectively, apart from pollutant removal from the wastewater (Kumar & Deswal 2021). In this study, biological treatment of rice mill wastewater has been performed using two anaerobic semi-continuous reactors (ASCR). The AD process involves microbes (obligate and facultative anaerobes) that grow in the absence of molecular oxygen and use organic substrates as food resources. The microbes metamorphose the organic substrate into oxidized matter (digestate), new bacterial cells, energy for their life cycles, and biogas comprising mainly biomethane (CH_4) and carbon dioxide (CO_2) gases.

Various research works have been performed on the RMWW treatment (Xin et al. 2018). The treated wastewater discharged from the various industrial wastewater treatment plants (IWWTP), still comprises some deleterious toxins and becomes harmful to various ecosystems. (Dhanasekar & Sasivarman 2016, Nain et al. 2015). Hence, an imperative need is to evolve a novel, high-performance, and cost-effective technique that can dexterously remediate industrial wastewater, including RMWW (Hu et al. 2018, Yuan et al. 2012). Anaerobic digestion (AD) is a rational, practicable, and cost-effective technique for remediating RMWW. Liu et al. (2018) recommended the AD process as an eco-friendly biological treatment process. The AD process has many merits over physicochemical treatment processes (Mao et al. 2015). The AD process curtails the use of the comprehensive land area, does not give rise to an obnoxious smell, and diminishes organic load and pathogenic microbes while generating biomethane and digestate to be used as organic fertilizers as the end products of microbial metabolism (Weng et al. 2014). AD process is cost-effective compared to other treatment technologies adopted for remediating RMWW because it yields biomethane and digestate as organic manure at the end of the biochemical reactions. In the AD system, biomethane gas can be generated from various agricultural and organic

Table 1: Recent research papers (2015-2023) on anaerobic digestion of rice mill wastewater (RMWW) and similar effluents.

S. No.	Title of papers	Conclusions	Reference
1.	Treatment of parboiled rice manufacturing wastewater using anaerobic fixed-film bed reactor packed with special media.	The study examined the anaerobic digestion of RMWW using an ASFBR at varied OLRs. Biopac media yielded BOD/COD removal (83.0%-92.7%)/(80.2%-89.0%), surpassing fugino spiral media (79.4%-90.6%)/(76.7%-86.1%). Biopac-packed reactors showed superior pollutant removal efficiency across all OLRs.	Giri & Satyanarayan (2015)
2.	Rice mill wastewater treatment by up-flow anaerobic sludge blanket reactor.	The examination utilized a UASB digester to treat RMWW across three phases at hydraulic retention times (HRTs) of 20 h, 14 h, and 10 h. COD removal efficiencies of 97%, 89%, and 86% were achieved in phases I, II, and III, respectively. Despite a lower HRT and higher OLR, the best operating condition achieved an 86% COD reduction, indicating OLR's influence on removal efficiency.	Saini et al. (2016)
3.	Robust performance of a novel anaerobic biofilm membrane bioreactor with mesh filter and carbon fiber (ABMBR) for low to high-strength wastewater treatment	The anaerobic biofilm membrane reactor (ABMR) with mesh filter and carbon fiber effectively remediated low to high-strength wastewater. It maintained a stable COD reduction efficiency of 95% and produced biomethane at 290 mL/g COD. The reactor showed resilience to organic load changes, with minimal membrane fouling and dominance of aceticlastic methanogens like Methanosaeta in the biofilm.	Li et al. (2017)
4.	Digestive performance of sludge with different crop straws in mesophilic anaerobic digestion.	The study investigated the anaerobic digestion of various crop straws with sludge under mesophilic conditions, focusing on hydrolytic behavior and methane production. Wheat straw exhibited the highest biomethane potential (BMP) at 0.462 L.g^{-1} VS, while corn cob had the lowest at 0.368 L.g^{-1} VS due to its hemicellulosic content. Corn cob digestion resulted in the highest volatile solids (VS) removal at 68.8%, surpassing corn, wheat, and rice straw-loaded digesters.	Chen et al. (2019)
5.	Comparative evaluation of methanogenesis suppression methods in microbial fuel cell during rice mill wastewater treatment.	The study explored RMWW treatment using microbial fuel cells (MFCs) and assessed methanogenesis suppression's impact on COD removal and bioelectricity. Air exposure led to the highest power density (656.10 mW/m^3), followed by ultrasonication (525.62 mW.m^{-3}), while heat treatment resulted in the lowest (312.43 mWm^{-3}). However, heat treatment exhibited the highest COD removal efficiency (85.22%).	Raychaudhuri & Behera (2020)
6.	Methane production test of the anaerobic sludge from rice parboiling industries with biodiesel glycerol from rice bran oil in Brazil.	The research explored glycerol's impact on biomethane generation from the anaerobic digestion of RMWW. Using four digesters with varying glycerol proportions, a 1% addition resulted in the highest biomethane production (945.23 mL) and COD removal (77%), which is attributed to the methanogenic bacteria's prolonged stationary phase.	Lourenco et al. (2021)
7.	Effect of rice winery wastewater as a co-substrate to enhance anaerobic digestion of molasses for methane production.	In this investigation, rice winery wastewater (RWWW) and molasses were anaerobically co-digested with various concentrations and increased OLR (1.875 to 13 g COD/L). The highest COD removal rate, 90.20 ± 0.44 to 92.65 ± 0.38%, and maximum methane contents of 69.14 ± 1.4% were observed at feeding OLR of 7 to 13 g COD.L^{-1} and at 3.75 g COD.L^{-1}, respectively. However, only molasses at OLR 18.5 g COD.L^{-1}d^{-1} negatively affected anaerobic digestion.	Khan et al. (2022)
8.	Sequential anaerobic-aerobic treatment of rice mill wastewater and simultaneous power generation in a microbial fuel cell.	In this research, the performance of two microbial fuel cells- one with a biological cathode (MFC_B) and the other with an abiotic cathode (MFC_A) treating RMWW anaerobically was compared. MFC_B exhibited lower power density (379.53 mW.m^{-3} vs. MFC_A's 791.72 mW.m^{-1}). However, MFC_B demonstrated higher COD removal efficiency (96.8% vs. MFC_A's 88.4%), highlighting its potential for efficiency.	Raychaudhuri & Behera (2023)

wastes viz. rice straw, fruits and vegetable wastes, food wastes, dairy farm effluents, slaughterhouse wastes, and high and medium-strength industrial wastewaters, etc. (Naik et al. 2022, Agrawal et al. 2023, Kesharwani & Bajpai 2020, Tsegaye et al. 2022, Kalantzis et al. 2023). Biomethane gas is a sustainable, renewable, and eco-friendly energy source having zero carbon footprint and contributing significantly to the circular economy. The research findings (conclusions) of the anaerobic digestion of rice mill wastewater and similar effluents recently carried out by various researchers during 2015-2023 are given in Table 1.

The RMWW comprises an immense amount of inorganic and organic pollutants. It comprises high chemical oxygen demand (COD), biochemical oxygen demand (BOD), total solids (TS), total organic solids (TOS), total dissolved solids (TDS), total suspended solids (TSS), total alkalinity (TA), total hardness (TH), and moderate volatile fatty acids (VFA), sodium (Na), potassium (K), lignin and phenol, etc. However, the concentration of pollutants in RMWW depends on the varieties of paddy soaked, the extent of parboiling, and additives used to improve the marketability of the produced rice. The rice millers usually add urea (NH_2CONH_2) and common salt (NaCl) in the soaking tank at the time of parboiling as additives to impart the glazing to rice and inhibit the boiling point of soaking water respectively, which aggravates the pollutant concentration of the RMWW (Pradhan & Sahu 2004). Transferring this wastewater without proper treatment into surface water bodies can harm freshwater and seawater ecosystems as well as onto land surfaces, deteriorate the terrestrial ecosystem, and eventually pollute the groundwater by contaminant transport. Hence, developing novel, affordable, efficient, environment-friendly, and sustainable techniques for remediating agro-industrial wastewater, including RMWW, is a prerequisite to achieving a sanitary environment.

The aim of this research is focused on the AD of RMWW in an anaerobic semi-continuous reactor (ASCR) with different OLR and HRT conditions by splitting the entire study into three operating phases, namely operating phase I (OP I): varying the HRT and keeping the OLR constant, operating phase II (OP II): varying both the OLR and HRT, and operating phase III (OP III): varying the OLR and keeping HRT constant, aimed at determining the most optimal conditions for biogas and biomethane generation as well as a substantial removal of the pollutants viz., BOD, COD, TS, TDS, TSS, TOS, lignin, and phenol, etc. from the RMWW. The novelty of this research paper is the substantial removal of the two potent biorefractory organics- lignin and phenol from the RMWW by White rot fungi (*Phanerochaete chrysosporium*), as well as lignin and phenol removing microbial species viz., *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Actinomycetes* sp., *Streptomycetes* sp. and *Aspergillus* sp. present in the cow-dung fed biogas plant sludge (BGPS), used as inoculum.

MATERIALS AND METHODS

Chemicals and Instruments Used

All chemicals and reagents of analytical grade (97 to 99% purity) were used for the experimental works. The glassware used was manufactured from borosilicate glass. The weight measurements during the conduction of experiments were done using the analytical balance present in the lab. The deionized water was used for analyses. The segregation and quantification of the constituents of the biogas (viz., CH_4 and CO_2) generated from bioreactors were performed with the aid of the Gas Chromatography (GC) Apparatus.

Additives Used

White rot fungi (*Phanerochaete chrysosporium*) and jaggery (*Saccharum officinarum*) were used as additives to the substrate (RMWW) of the lab-scale bioreactors. *Phanerochaete chrysosporium* was used to degrade the biorefractory organics lignin and phenol, and it was collected from the Biotechnology Department, Pandit Ravishankar Shukla University, Raipur, Chhattisgarh, India. Jaggery was used to enhance the metabolism of the microorganisms present in the BGPS. It was purchased from the local market of Raipur.

Properties of Jaggery

Jaggery is rich in carbon and energy. It stimulates the metabolic activity of the bacteria and boosts the reproduction of new bacterial cells by cell division, thereby enhancing the bacterial growth rate in the anaerobic reactors. It balances the dehydrogenase activity, especially in case of temperature drop in the winter season, and facilitates the smooth functioning of the bioreactor (Aralkar et al. 2023). The dehydrogenase activity is associated with the anaerobic biodegradability of organic compounds by microbial activity. It can be a good indication of the microorganism activity (Hongwei et al. 2002). In the lab-scale anaerobic digester, the biogas production was increased by 36.8% when cattle dung (3.00 kg) was supplemented by 30 g of jaggery @10 g jaggery/kg of cow dung (Masih et al. 2019). The physicochemical properties and nutritional content of jaggery are shown in Table 2.

Collection of RMWW Sample and its Characterization

The RMWW samples were collected from a nearby rice mill for feeding in reactors and their physicochemical analyses.

Table 2: Physicochemical characteristics and nutritional content of Jaggery.

S. No.	Parameters	Unit	Range of Values
1.	pH	-	5.8-6.4
2.	Density	$g.cm^{-3}$	1.5
3.	Viscosity	Centipoise[cP]	807
4.	Melting point	K	460
5.	Molecular mass	$g.mol^{-1}$	342
6.	Carbohydrate	%	83.90-97.20
7.	Reducing sugar	%	10.50
8.	Total sugar	%	87.50-95.40
9.	Sucrose	%	76.55-89.48
10.	Iron [Fe]	$\mu g.mL^{-1}$	1.60-2.50
11.	Copper [Cu]	$\mu g.mL^{-1}$	0.17-8.50
12.	Zinc (Zn)	$\mu g.mL^{-1}$	0.10-1.76
13.	Cobalt [Co]	$\mu g.mL^{-1}$	9.90
14.	Manganese [Mn]	$\mu g.mL^{-1}$	0.35-1.66
15.	Iodine [I]	$\mu g.mL^{-1}$	0.01

(Aralkar et al. 2023, Sharifi-Rad et al. 2023)

Their physicochemical characteristics were determined in the Environmental Engineering Lab, NIT Raipur, as per the standard methods by APHA, AWWA, and WWF (2017) 23rd Edition, except for the CHNSO analysis (C/N ratio of dry RMWW) performed at Pt. Ravi Shankar Shukla University, Raipur. Fifteen RMWW samples were collected on different days and seasons- monsoon, pre-monsoon, post-monsoon, summer, and winter from January 2022 to March 2023. The samples were taken in low-density polyethylene (LDPE) bottles of 1.0-liter (L) capacity each. Three LDPE bottles of 1.0 L capacity were used for each sampling. The first bottle was filled with absolute RMWW for determining pH, EC, turbidity, sulfate, chloride, lignin, BOD, TS, TOS, TIS, TDS, TSS, DO, TA, TH, Ca-hardness, Mg-hardness, and VFA. The second bottle was filled with RMWW acidified with Conc. H_2SO_4 for determining COD, total Kjeldahl nitrogen (TKN), nitrate (NO_3), phosphate (PO_4), oil and grease, phenol (C_6H_5OH), etc. The third bottle was filled

Table 3: Physicochemical characteristics of RMWW.

S. No.	Parameters	Unit	Range of Values	Mean value
1.	pH	-	5.28 - 6.08	5.64
2.	Color	-	Faint brown to Faint yellow	-
3.	Electrical Conductivity [EC]	$\mu S.cm^{-1}$	5.65 - 7.50	6.84
4.	Turbidity	NTU	39.4 - 43.8	40.5
5.	Total Alkalinity [TA]	$mg.L^{-1}$ as $CaCO_3$	2,251 - 2,467	2,356
6.	Total Hardness [TH]	$Mg.L^{-1}$ as $CaCO_3$	2,320 - 2,537	2,440
7.	Calcium Hardness [Ca- H]	$mg.L^{-1}$ as $CaCO_3$	1,833 - 1,964	1,906
8.	Mg- Hardness (Mg-H)	$mg.L^{-1}$ as $CaCO_3$	487 – 573	534
9.	Chemical Oxygen Demand [COD]	$mg.L^{-1}$	5,145 - 5,427	5,274
10.	Biochemical Oxygen Demand [BOD]	$mg.L^{-1}$	3,410 - 3,785	3,612
11.	Total Kjeldahl Nitrogen [TKN]	$mg.L^{-1}$	45 – 65	54
12.	Total Solids (TS)	$mg.L^{-1}$	3,837 - 4,200	4,078
13.	Total Organic Solids [TOS]	$mg.L^{-1}$	2,847 - 3,180	3,068
14.	Total Inorganic Solids [TIS]	$mg.L^{-1}$	990 - 1,020	1,010
15.	Total Dissolved Solids [TDS]	$mg.L^{-1}$	2,733 - 3,060	2,958
16.	Total Suspended Solids [TSS]	$mg.L^{-1}$	1104 - 1,140	1120
17.	Dissolved Oxygen [DO]	$mg.L^{-1}$	0.9 - 2.0	1.5
18.	Chloride [Cl^-]	$mg.L^{-1}$	673 – 850	746
19.	Sulfate [SO_4^{3-}]	$mg.L^{-1}$	61 – 78	71
20.	Oil & Grease	$mg.L^{-1}$	14 – 24	18
21.	Nitrate [NO_3^-]	$mg.L^{-1}$	19 -29	23
22.	Orthophosphate [PO_4^{3-}]	$mg.L^{-1}$	82 – 94	87
23.	Lignin	$mg.L^{-1}$	122 - 141	138
24.	Phenol [C_6H_5O]	$mg.L^{-1}$	12 -20	16
25.	Sodium [Na]	$mg.L^{-1}$	70 – 82	79
26.	Potassium [K]	$mg.L^{-1}$	450 – 550	496
27.	Volatile Fatty Acids [VFA]	$mg.L^{-1}$	290 – 350	318
28.	VFA/Alkalinity ratio	-		0.13
29.	Carbon	%		22.449
30.	Nitrogen	%		1.821
31.	Carbon/Nitrogen [C/N] ratio	-		12.3

Table 4: Physicochemical characteristics of BGPS.

S. No.	Parameters	Unit	Mean value
1.	pH	-	7.5
2.	Color	-	Black
3.	Carbon	%	32.904
4.	Nitrogen	%	2.297
5.	C/N ratio	-	14.3
6.	VFA	$mg.L^{-1}$	115
7.	Alkalinity	$mg.L^{-1}$ as $CaCO_3$	2675
8.	VFA/Alkalinity ratio	-	0.04
9.	COD	$mg.L^{-1}$	21,695

with RMWW acidified with Conc. HNO_3 for determining sodium (Na) and potassium (K). All bottles filled with wastewater samples were preserved in the Lab refrigerator at 4°C temperatures to maintain the sample's integrity. The wastewater for feeding in anaerobic reactors was also collected from the same rice mill in three plastic cans of 10 L capacity each. The physicochemical parameters of each sample were analyzed in triplicate and their mean value was taken. The range of values and the mean value of each parameter are shown in Table 3.

Inoculum Used for Reactors

Biogas plant sludge (BGPS) was used as inoculum for the reactors. It was taken from the biogas plant operated by Sarda Dairy (Vachan Milk) Limited, Kharora, Chhattisgarh, India. It is a cow dung-fed biogas plant of a 2000 m^3 capacity with two anaerobic digesters- each producing 1000 m^3 of biogas daily. The physicochemical examination of BGPS was done as per the standard methods by APHA in the Lab, except for the CHNSO analysis (C/N ratio of BGPS) that was done at IIT Bombay. The physicochemical characteristics of BGPS are shown in Table 4.

Experimental Set-up for Anaerobic Digestion of RMWW

Two anaerobic semi-continuous reactors (ASCR)- R1 and R2 of 35 L capacity each and made of high-density polyethylene (HDPE) provided with feed inlets on their top for feeding the substrate (RMWW) and inoculum (BGPS), were taken for AD of the wastewater. In both reactors, 24 L of RMWW (80%) and 6 L of BGPS (20%), i.e., 30 L of the mix along with 24 g jaggery @ 1 g jaggery/L and 24 g white-rot fungi (*Phanerochaete chrysosporium*) @ 1 g fungi/L were fed from their feed inlets. The substrate pH of reactors R1 and R2 were adjusted to 7.0 by adding 24 g NaOH@ 1 g $NaOH.L^{-1}$ and 12 g $Ca(OH)_2$@ 1 g $Ca(OH)_2.L^{-1}$, respectively. The clearance volume left in both reactors above the substrate and inoculum mix was 35 L-30 L= 5 L, which was left for the biogas released in the process of AD. The biogas produced in both reactors was volumetrically measured by the water displacement techniques in the inverted measuring cylinders of 1 L capacity each. In both reactors, manual feeding was accomplished in semi-continuous mode through their feed inlets. The effluent samples for analyses were collected from taps provided in both reactors- R1 and R2. The blending of substrate, co-substrate, and inoculum was performed manually before feeding the reactors. The anaerobic reactors- R1 and R2 were kept in the ambient atmosphere at mesophilic

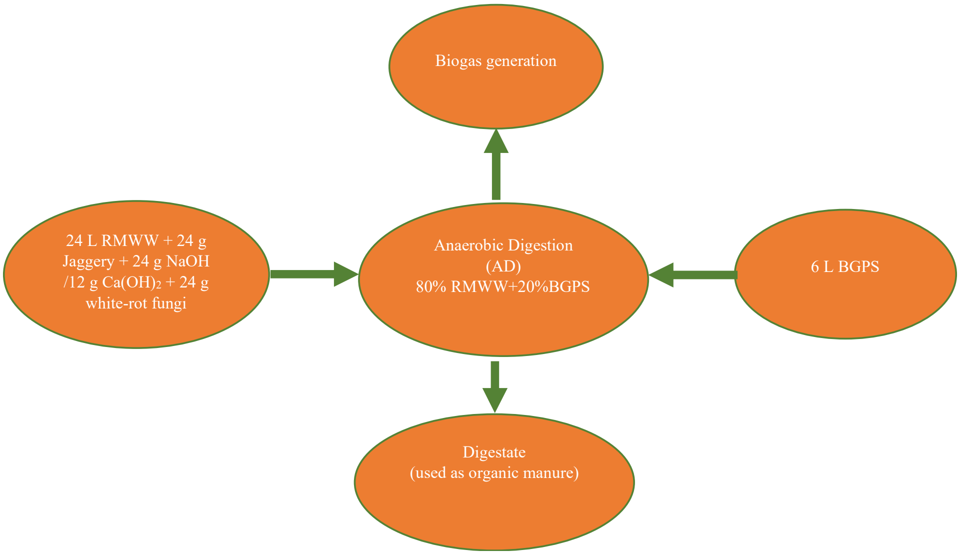


Fig. 2: Schematic diagram of biogas generation from AD of RMWW.

temperatures (18°C to 42°C). The biogas generation began 16 days after the date of feeding the reactors. After attaining constant and steady biogas generation for consecutive 4 days, the organic loading was started, and the biogas generation was recorded daily. Fig. 2 depicts a schematic diagram of biogas generation from anaerobic digestion of rice mill wastewater. The cumulative sum of daily biogas generation from both reactors was found every 10 days after the end of each HRT. Furthermore, BOD, COD, TS, TOS, TDS, TSS, lignin, and phenol contents of the reactors' substrate (RMWW) were determined, which were 3725, 5425, 4194, 3177,3059,1135, 135, and 19 mg.L^{-1} respectively at the time of loading the reactors- R1 and R2. The organic loading capacity of both reactors was 7.50 kg COD.m^{-3}.d^{-1} each.

The stationary condition of the bioreactors- R1 and R2 was maintained for the subsequent 16 days for stabilization of the substrate. Fig. 3a and 3b exhibit the bioreactors- R1 and R2 used for the experimental analyses delineating the feed inlets for loading substrate into the reactors as well as gas pipes, gas valves, and effluent collection taps for collecting biogas and reactor effluent. The biogas generated from the reactors R1 and R2 were conveyed through the gas pipes and collected in the inverted measuring cylinders. Fig. 4 shows additives used in anaerobic reactors- R1 and R2.

Operating Phases of Anaerobic Reactors- R1 and R2

After 16 days of stabilization of the anaerobic bioreactors- R1 and R2, the biogas generation commenced. After confirming the stabilization, the OLR was initiated after 4 d of the arrival of steady biogas production, and every OLR was capered to the upcoming loading after safeguarding 9 d of consistent biogas generation. The research was split into three operating phases, namely operating phase I (impact of changing HRT and keeping OLR constant), operating phase II (impact

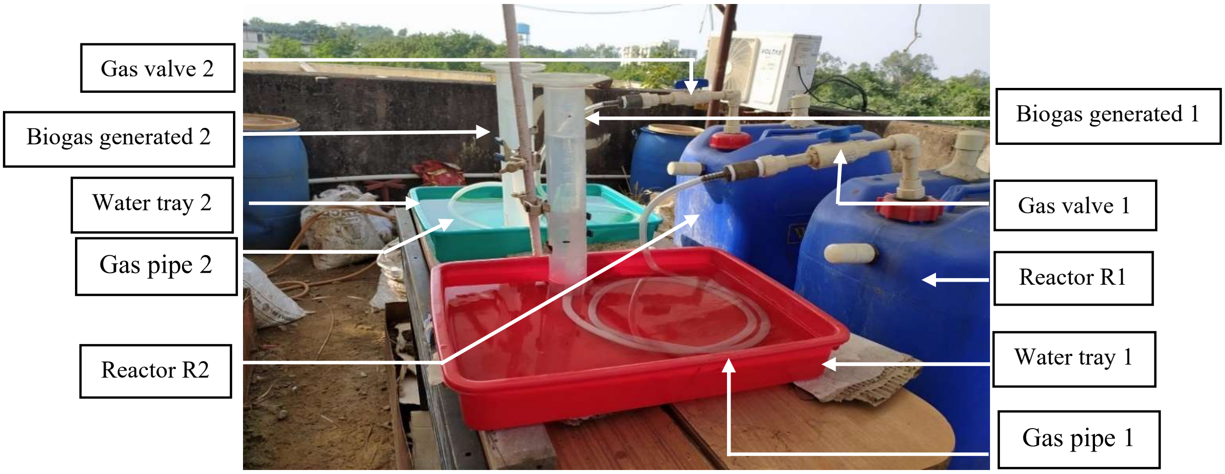


Fig. 3a: Lab-scale anaerobic reactors R1 and R2 showing biogas.

Fig. 3b: Lab-scale anaerobic reactors R1 and R2 showing feed inlets and effluent collection taps.

Jaggery

Calcium hydroxide

Sodium hydroxide

White-rot fungi

Fig. 4: $Ca(OH)_2$, NaOH, Jaggery, and White-rot fungi used as additives in reactors- R1 and R2.

of changing both OLR and HRT), and operating phase III (impact of changing OLR and keeping HRT constant) based on enhancing and diminishing the influent organic load (Veluchamy & Kalamdhad 2017). The biogas generation was monitored daily based on the HRT and OLR conditions, and the pollutants (BOD, COD, lignin, phenol, TS, TSS, TOS, and TDS) removal efficiencies of both reactors were found. The bioreactors- R1 and R2 were run for a total duration of 370 d in three operating phases, as mentioned below:

Operating Phase I (OP I): In this phase, OLR remained constant at 3.25 kg $COD.m^{-3}.d^{-1}$, and HRT was varied from 10 d to 110 d. The bioreactors' start-up time was 16 d from the date of feeding, and their smooth functioning, along with the invariable biogas production, commenced 4 d after the start-up time. As a result, the OLR was started after 20 d (16 d+4 d), i.e., after the constant and steady biogas generation. Thus, the total days of bioreactors' run in OP I = 110 + 20 = 130 d.

Operating Phase II (OP II): In OP II, OLR was varied from 1.50 to 7.32 kg $COD.m^{-3}.d^{-1}$, and HRT was varied from 10 d to 150 d. Thus, the total days of the bioreactors' run in this phase, including the commencement of the smooth functioning of the bioreactors and constant and steady biogas generation = 150 d + 20 d = 170 d.

Operating Phase III (OP III): In OP III, OLR was varied from 1.55 to 7.25 kg $COD.m^{-3}.d^{-1}$ in 10 segments, and HRT was kept constant for each OLR at 5 d. Thus, the total days of the bioreactors' run in this phase, including the commencement of the smooth functioning of the bioreactors and constant and steady biogas generation = 50 d + 20 d = 70 d.

Hence, the total duration of the bioreactors' run in all three phases = 130 d +170 d + 70 d = 370 d.

Effluent Analyses and Biogas Measurement

In OP I, the effluents were collected from reactors R1 and R2 after an HRT of 10 d, 20 d, 30 d, 40 d, 50 d, 60 d, 70 d, 80 d, 90 d, 100 d, and 110 d for the physicochemical analyses with the help of the effluent collection taps provided in them as shown in Fig. 3b, when OLR was kept constant at 3.25 kg $COD.m^{-3}.d^{-1}$. In OP II, the effluents were collected from the reactors after an HRT of 10 d, 20 d, 30 d, 40 d, 50 d, 60 d, 70 d, 80 d, 90 d, 100 d, 110 d, 120 d, 130 d, 140 d, and 150 d for analyses, when OLR was varied from 1.50 to 7.32 kg $COD.m^{-3}.d^{-1}$. Eventually, in OP III, effluents were collected from the reactors after a constant HRT of 5 d each time, when OLR was varied from 1.55 to 7.25 kg $COD.m^{-3}.d^{-1}$. The pH, VFA, Alkalinity, BOD, COD, lignin, phenol, TS, TSS, TOS, and TDS of the effluents collected in each operating phase after each HRT from reactors R1 and R2 were determined in the Lab as per the standard methods by APHA, AWWA, and WWF (2017) 23rd Edition. The VFA/Alkalinity ratio and pH of the reactors' substrate were regularly monitored after each HRT in all three phases. The values were found within permissible limits each time, which reflects that both reactors were operating satisfactorily in all three phases. Pollutants (BOD, COD, TS, TSS, TOS, TDS, lignin, and phenol) removal percentages of the effluents were determined after each HRT to know the comparative performances of both reactors. The volumes of biogas generated from both reactors- R1 and R2 were separately measured by the inverted measuring cylinders by the water displacement techniques, as shown in Fig. 3a. The biogas generated from both reactors mainly comprised CH_4 and CO_2, which were 51% and 49% respectively as analyzed by Gas Chromatography (GC).

RESULTS AND DISCUSSION

Impact of OLR and HRT Variations

Organic Loading Rate (OLR): It expresses the amount of suspended and dissolved organic materials fed into the reactor per cubic meter of its volume per day. It is expressed in kg $COD.m^{-3}.d^{-1}$ or g $COD.L^{-1}.d^{-1}$. The organic loading is done in a semi-continuous mode in this study. When OLR is increased, the volume of the biogas production is increased to an appreciable extent. However, the reactor's performance and equilibrium of the biochemical process are degraded.

Hydraulic Retention Time (HRT): The average retention time for which wastewater is retained in the bioreactor is referred to as HRT. It is expressed in day (d). The HRT depends upon OLR, process temperature, substrate characteristics, and microbial growth rate.

In this investigation, the mesophilic anaerobic degradation of RMWW was carried out in the ambient atmospheric conditions of NIT Raipur in changing OLR and HRT conditions. The pH, VFA, alkalinity, and VFA/Alkalinity ratio, as well as BOD, COD, lignin, phenol, TS, TSS, TOS, and TDS removal of the effluents from both reactors, were examined by the optimization of OLR and HRT conditions.

Experimental Outcomes

Tables 5a, 5b, 5c, and 6a, 6b, 6c illustrate the experimental findings of RMWW treatment in three operating phases using two anaerobic semi-continuous reactors (ASCR)- R1 and R2, respectively, in which the pH adjustment of the substrate to 7.0 is done by blending NaOH and $Ca(OH)_2$ respectively with the substrate. In Operating Phase I, the OLR was kept constant in both reactors R1 and R2 at 3.25 kg $COD.m^{-3}.d^{-1}$ for the entire HRT (10 d to 110 d), and pollutant removal, as well as biogas generation, was inspected under varying HRT conditions. In Operating Phase II, the HRT was changed from 10 d to 150 d, and the OLR was changed from 1.50 to 7.32 kg $COD.m^{-3}.d^{-1}$. In Operating Phase III, the HRT was kept constant at 5 d, and the OLR was changed from 1.55 to 7.25 kg $COD.m^{-3}.d^{-1}$. The pollutant (BOD, COD, lignin, phenol, TS, TSS, TOS, and TDS) removal and biogas generation from both reactors were examined in all three operating phases. The highest BOD removal percentages achieved in reactors R1 and R2 were 94% and 93% respectively, in Operating Phase I of both reactors, shown in Tables 5a and 6a, respectively.

Behavior of pH, VFA, and Alkalinity in Different Operating Phases

Fig. 5 illustrates the pH variation in the biological treatment

Table 5a: Operating Phase I: Varying HRT from 10 d to 110 d and keeping OLR constant at 3.25 kg $COD.m^{-3}.d^{-1}$ (R1).

HRT [d]	10	20	30	40	50	60	70	80	90	100	110
OLR [g $COD.m^{-3}.d^{-1}$]						3.25					
BOD removal [%]	65	76	85	94	93	94	94	93	93	94	94
COD removal [%)]	62	74	82	92	91	92	92	91	91	92	92
Lignin removal [%]	39	51	68	84	83	84	84	83	83	84	84
Phenol removal [%]	36	49	65	82	81	82	82	81	81	82	82
TSS removal [%]	56	65	75	88	87	88	88	87	87	88	88
TOS removal [%]	58	69	79	92	91	92	92	91	91	92	92
TDS removal [%]	58	71	81	92	91	92	92	91	91	92	92
Biogas generation [$L.d^{-1}$]	07.51	07.81	08.32	08.52	08.51	08.50	08.51	08.51	08.49	08.49	08.50
Biogas yield [$L.g^{-1}$ COD]	0.007	0.007	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Biomethane generation ($L.d^{-1}$)	03.81	03.92	04.21	04.32	04.32	04.31	04.32	04.32	04.31	04.31	04.31
Biomethane yield ($L.g^{-1}$ COD)	0.003	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
pH	7.6	7.7	7.6	7.5	7.4	7.3	7.2	7.1	7.1	7.0	6.9
VFA [$mg.L^{-1}$ as CH_3COOH]	85	82	78	73	72	65	59	54	47	44	40
Alkalinity [$mg.L^{-1}$ as CaCO]	894	882	877	861	852	830	795	786	760	750	734
VFA/Alkalinity ratio	0.09	0.09	0.09	0.08	0.08	0.08	0.07	0.07	0.06	0.06	0.05

Table 5b: Operating Phase II: Varying OLR from 1.50 to 7.32 kg COD.m^{-3}.d^{-1} and HRT from 10 d to 150 d (R1).

HRT [d]	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
OLR [g COD.m$^{-3}\cdot$d$^{-1]}$	1.50	2.50	3.25	3.75	4.25	4.75	5.25	5.50	5.75	6.00	6.25	6.50	6.75	7.00	7.32
BOD removal [%]	92	91	90	89	87	85	83	82	81	80	78	76	74	73	71
COD removal [%)]	90	89	88	87	86	84	82	81	80	78	77	75	73	72	70
Lignin removal [%]	82	80	78	76	74	73	71	69	68	66	64	61	59	57	54
Phenol removal [%]	80	77	76	74	73	71	69	66	65	64	62	59	56	55	52
TSS removal [%]	89	88	87	86	85	84	82	81	80	79	77	75	73	71	70
TOS removal [%]	86	85	84	83	82	81	79	78	77	76	74	73	71	69	68
TDS removal [%]	91	90	89	88	87	86	84	83	82	81	79	76	73	72	71
Biogas generation [L.d^{-1}]	90	89	88	87	86	85	83	82	81	80	78	75	73	71	70
Biogas yield [L.g^{-1} COD]	04.06	07.02	08.71	10.50	10.42	11.21	12.03	12.06	12.11	12.06	12.63	12.62	12.61	12.62	12.61
Biomethane generation (L.d^{-1})	0.009	0.009	0.009	0.010	0.008	0.008	0.008	0.007	0.007	0.007	0.007	0.007	0.006	0.006	0.005
Biomethane yield (L.g^{-1} COD)	02.06	03.51	04.41	05.32	05.35	05.71	06.11	06.21	06.21	06.22	06.40	06.40	06.40	06.40	06.40
pH	0.004	0.004	0.005	0.005	0.004	0.004	0.004	0.004	0.004	0.003	0.004	0.004	0.003	0.003	0.002
VFA [mg.L^{-1} as CH$_3$COOH]	7.8	7.7	7.8	7.6	7.7	7.8	7.7	7.6	7.5	7.4	7.5	7.6	7.3	7.5	7.3
Alkalinity [mg.L^{-1} as CaCO]	94	90	83	79	75	72	69	66	61	57	55	52	49	44	37
VFA/Alkalinity ratio	887	884	881	876	870	864	860	856	852	849	842	838	830	824	790
	0.10	0.10	0.09	0.09	0.08	0.08	0.08	0.08	0.07	0.07	0.06	0.06	0.06	0.05	0.04

Table 5c: Operating Phase III: Varying OLR from 1.55 to 7.25 kg COD.m^{-3}.d^{-1} and keeping HRT constant at 5 d (R1).

HRT [d]	5									
OLR [g COD.m$^{-3}\cdot$d$^{-1]}$	1.55	2.55	3.05	3.55	4.05	4.55	5.05	5.55	6.05	7.25
BOD removal [%]	80	77	75	71	69	66	64	62	59	55
COD removal [%)]	78	75	73	69	67	64	62	60	57	53
Lignin removal [%]	69	66	62	58	53	47	40	33	28	17
Phenol removal [%]	66	63	59	55	49	42	37	29	22	14
TSS removal [%]	77	74	72	68	66	63	61	59	56	53
TOS removal [%]	74	71	69	65	63	60	58	56	53	50
TDS removal [%]	79	76	74	70	68	65	63	62	58	53
Biogas generation [L.d^{-1}]	78	75	73	69	67	64	62	60	57	54
Biogas yield [L.g^{-1} COD]	0.387	0.535	0.549	0.639	0.729	0.819	0.909	0.999	0.907	1.087
Biomethane generation (L.d^{-1})	0.008	0.007	0.006	0.006	0.006	0.006	0.006	0.006	0.005	0.005
Biomethane yield (L.g^{-1} COD)	0.197	0.273	0.281	0.326	0.372	0.417	0.463	0.509	0.462	0.554
pH	0.004	0.004	0.004	0.003	0.003	0.003	0.003	0.002	0.002	0.002
VFA [mg.L^{-1} as CH$_3$COOH]	7.7	7.6	7.6	7.5	7.4	7.3	7.2	7.1	7.0	6.9
Alkalinity [mg.L^{-1} as CaCO]	84	81	78	74	71	67	62	59	52	48
VFA/Alkalinity ratio	878	870	860	845	796	784	764	748	724	712
	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.07	0.07

Table 6a: Operating Phase I: Varying HRT from 10 d to 110 d and keeping OLR constant at 3.25 kg COD.m^{-3}.d^{-1} (R2).

HRT [d]	10	20	30	40	50	60	70	80	90	100	110
OLR [g COD.m$^{-3\cdot}$d$^{-1]}$						3.25					
BOD removal [%]	64	75	84	93	92	93	93	92	92	93	93
COD removal [%)]	61	73	81	91	90	91	91	90	90	91	91
Lignin removal [%]	37	49	65	82	81	82	82	81	81	82	82
Phenol removal [%]	34	47	62	80	79	80	80	79	79	80	80
TSS removal [%]	56	67	78	90	89	90	90	89	89	90	90
TOS removal [%]	53	63	74	87	86	87	87	86	86	87	87
TDS removal [%]	57	68	78	91	90	91	91	90	90	91	91
Biogas generation [L.d^{-1}]	57	69	80	91	90	91	91	90	90	91	91
Biogas yield [L.g^{-1} COD]	07.47	07.73	08.21	08.51	08.50	08.50	08.49	08.50	08.50	08.49	08.49
Biomethane generation (L.d^{-1})	0.007	0.007	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008
Biomethane yield (L.g^{-1} COD)	03.81	03.93	04.17	04.33	04.31	04.32	04.31	04.32	04.32	04.31	04.31
pH	0.003	0.003	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004
VFA [mg.L^{-1} as CH_3COOH]	7.7	7.8	7.7	7.6	7.5	7.4	7.3	7.2	7.2	7.1	7.0
Alkalinity [mg.L^{-1} as CaCO]	89	84	80	75	73	68	61	55	49	45	42
VFA/Alkalinity ratio	897	884	880	865	854	832	798	785	765	754	736
	0.10	0.09	0.09	0.08	0.08	0.08	0.07	0.07	0.06	0.06	0.06

Table 6b: Operating Phase II: Varying OLR from 1.50 to 7.32 kg COD.m^{-3}.d^{-1} and HRT from 10 d to 150 d (R2).

HRT [d]	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
OLR [g COD.m$^{-3\cdot}$d$^{-1]}$	1.50	2.50	3.25	3.75	4.25	4.75	5.25	5.50	5.75	6.00	6.25	6.50	6.75	7.00	7.32
BOD removal [%]	91	90	89	88	86	84	82	81	80	79	77	75	74	72	70
COD removal [%)]	89	88	87	86	85	83	81	80	79	77	76	74	73	71	69
Lignin removal [%]	80	78	76	74	72	70	68	67	66	64	62	59	57	55	51
Phenol removal [%]	76	74	72	70	68	67	65	64	62	60	58	56	55	53	49
TSS removal [%]	88	87	86	85	84	83	81	80	79	78	76	74	72	70	69
TOS removal [%]	85	84	83	82	81	80	78	77	76	75	73	72	70	68	67
TDS removal [%]	90	89	88	87	86	85	83	82	81	80	78	75	72	71	70
Biogas generation [L.d^{-1}]	89	88	87	86	85	84	82	81	80	79	77	74	72	70	69
Biogas yield [L.g^{-1} COD]	04.06	07.02	08.72	10.51	10.41	11.21	12.02	12.06	12.11	12.06	12.62	12.61	12.60	12.61	12.60
Biomethane generation (L.d^{-1})	0.009	0.009	0.009	0.010	0.008	0.008	0.008	0.007	0.007	0.007	0.007	0.007	0.006	0.005	0.005
Biomethane yield (L.g^{-1} COD)	02.06	03.51	04.41	05.31	05.31	05.71	06.11	06.21	06.21	06.21	06.41	06.40	06.39	06.40	06.39
pH	0.004	0.004	0.005	0.005	0.004	0.004	0.004	0.004	0.004	0.003	0.004	0.004	0.003	0.003	0.003
VFA [mg.L^{-1} as CH_3COOH]	7.9	7.8	7.9	7.8	7.7	7.9	7.7	7.6	7.5	7.4	7.5	7.4	7.6	7.5	7.4
Alkalinity [mg.L^{-1} as CaCO]	98	95	85	81	78	75	72	69	63	59	56	54	51	45	39
VFA/Alkalinity ratio	890	886	883	879	872	865	863	858	855	850	844	840	834	828	795
	0.11	0.11	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.07	0.07	0.06	0.06	0.05	0.05

Table 6c: Operating Phase III: Varying OLR from 1.55 to 7.25 kg COD.m^{-3}.d^{-1} and keeping HRT constant at 5 d (R2).

HRT [d]					5					
OLR [g COD.m^{-3}·d$^{-1]}$	1.55	2.55	3.05	3.55	4.05	4.55	5.05	5.55	6.05	7.25
BOD removal [%]	79	76	74	70	68	65	63	61	58	54
COD removal [%)]	77	74	72	68	66	63	61	59	56	52
Lignin removal [%]	67	64	60	55	52	45	38	29	24	15
Phenol removal [%]	64	61	57	54	45	40	35	26	19	11
TSS removal [%]	76	73	71	67	65	62	60	58	55	52
TOS removal [%]	73	70	68	64	62	59	57	55	52	49
TDS removal [%]	78	75	73	69	67	64	62	61	57	52
Biogas generation [L.d^{-1}]	77	74	72	68	66	63	61	59	56	53
Biogas yield [L.g^{-1} COD]	0.383	0.607	0.697	0.773	0.847	0.860	0.903	0.940	0.963	1.013
Biomethane generation (L.d^{-1})	0.008	0.008	0.008	0.007	0.007	0.006	0.006	0.006	0.005	0.004
Biomethane yield (L.g^{-1} COD)	0.195	0.309	0.353	0.393	0.430	0.437	0.460	0.477	0.490	0.517
pH	0.004	0.004	0.004	0.004	0.003	0.003	0.003	0.003	0.003	0.002
VFA [mg.L^{-1} as CH_3COOH]	7.8	7.7	7.7	7.6	7.5	7.4	7.3	7.2	7.1	7.0
Alkalinity [mg.L^{-1} as CaCO]	88	85	80	77	73	69	65	61	55	51
VFA/Alkalinity ratio	884	875	866	850	798	786	767	754	729	716
	0.10	0.10	0.09	0.09	0.09	0.09	0.08	0.08	0.07	0.07

of RMWW in ASCR- R1 and R2. The pH of the substrate and inoculum mix in both reactors was regularly monitored. As shown in Fig. 5a, pH values in reactors R1 and R2 were 7.7 and 7.8, respectively, at an HRT of 20 d in OP I, and they gradually diminished in both reactors with the increase in HRT. Similarly, Fig. 5b and 5c illustrate the pH range of OP II and OP III, respectively. In OP II, the highest pH values in reactors R1 and R2 were 7.8 and 7.9, respectively, which were achieved under three conditions- an HRT of 10 d and OLR of 1.50 kg COD.m^{-3}.d^{-1}, HRT of 30 d and OLR of 3.25 kg COD.m^{-3}.d^{-1}, and HRT of 60 d and OLR of 4.75 kg COD.m^{-3}.d^{-1}. In OP III, the highest values of pH in reactors R1 and R2 were 7.7 and 7.8, respectively, at an OLR of 1.55 kg COD.m^{-3}.d^{-1}, and they gradually diminished with the increasing OLR in both reactors.

The analysis of VFA variation was done in three operating phases based on increase and decrease in feed concentration; the results are depicted in Fig. 6a, 6b, and 6c. During acetogenic conditions, VFA and alcohols are converted into acetic acid by H_2-utilizing homo-acetogens and H_2+CO_2 by H_2-producing acetogens, which are ultimately converted to methane and CO_2 gas by acetoclastic and CO_2-reducing methanogens respectively. As a result, pH is not lowered below 6.5 throughout the digestion process in

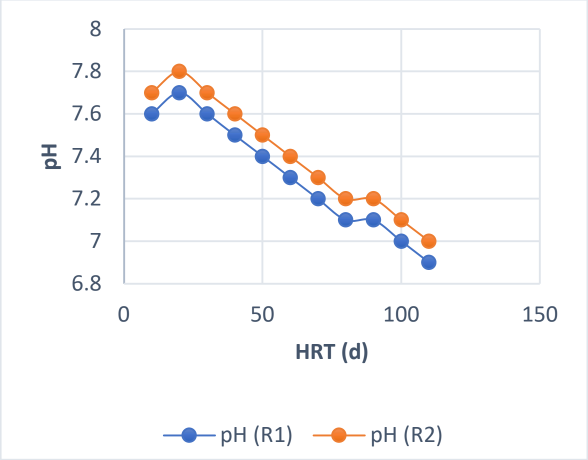


Fig. 5a: pH variation with HRT in OP I.

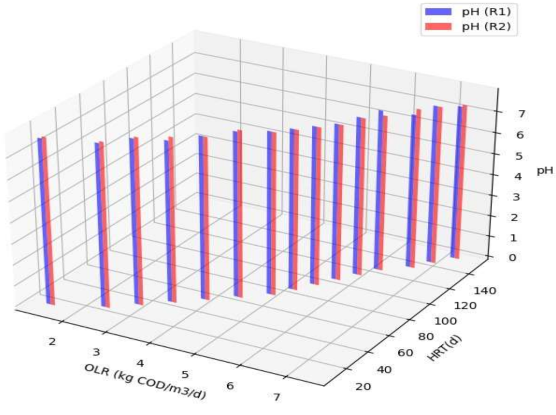


Fig. 5b: pH variation with varying HRT and OLR in OP II.

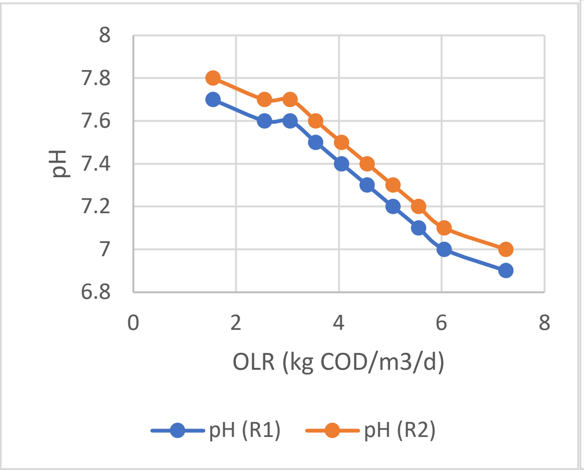


Fig. 5c: pH variation with OLR in OP III.

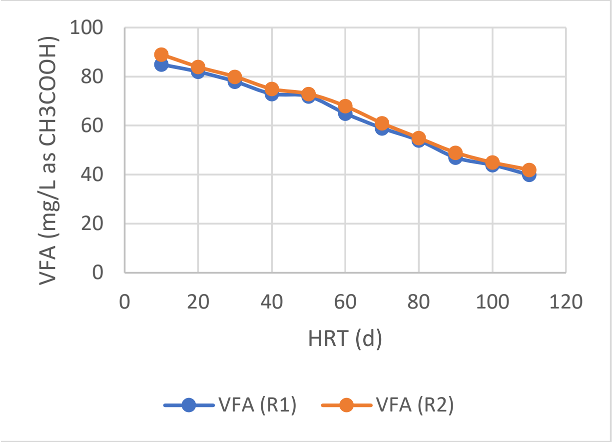


Fig. 6a: VFA variation with HRT in OP I.

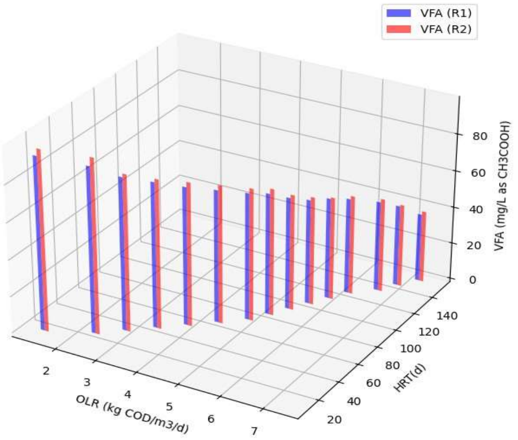


Fig. 6b: VFA variation with varying OLR and HRT in OP II.

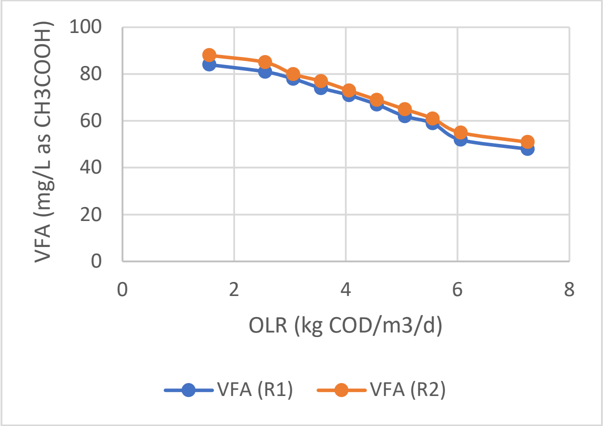


Fig. 6c: VFA variation with OLR in OP III.

any operating phase. The analysis of alkalinity variation was done in three operating phases, and the results are depicted in Fig. 7a, 7b, and 7c. The alkalinity of the RMWW was in the range of 712-897 mg.L^{-1} as $CaCO_3$ in the two reactors- R1 and R2. The alkalinity of RMWW in reactor R1 ranged from 734-894, 790-887, and 712-878 mg.L^{-1} as $CaCO_3$ in operating phases I, II, and III, respectively. Similarly, the alkalinity of RMWW in reactor R2 ranged from 736-897, 795-890, and 716-884 mg.L^{-1} as $CaCO_3$ in operating phases I, II, and III, respectively. Thus, the alkalinity of RMWW in both reactors did not fall below 712 mg.L^{-1} as $CaCO_3$ due to the conversion of VFA into CH_4 and CO_2.

Pollutant Reduction Efficiencies of ASCR- R1 and R2

BOD reduction efficiency: The BOD reduction efficiencies of the bioreactors R1 and R2 in operating phases I, II, and III are depicted in Fig. 8a, 8b, and 8c, respectively. Fig. 8a shows that at an HRT of 40 d, the BOD removal efficiencies of reactors R1 and R2 were 94% and 93%, respectively, in OP I when OLR was kept constant at 3.25 kg COD.m^{-3}.d^{-1}. In this phase, the BOD reduction efficiencies of the two reactors went on increasing from an HRT of 10 d up to 40 d, and at 40 d the maximum removal efficiencies of both reactors were observed. After 40 d up to 110 d, the BOD removal efficiencies of both reactors remained nearly stationary. Fig. 8b shows that the highest BOD removal efficiencies achieved in reactors R1 and R2 were 92% and 91%, respectively, at an OLR of 1.50 kg COD.m^{-3}.d^{-1} and HRT of 10 d in OP II. Similarly, in OP III, the highest BOD removal efficiencies attained in reactors R1 and R2 were 80% and 79%, respectively, at the OLR of 1.55 kg COD.m^{-3}.d^{-1} and constant HRT of 5 d, as illustrated in Fig. 8c. In this phase, BOD removal efficiencies were decreased as the OLR was increased from 1.55 kg COD.m^{-3}.d^{-1}. Thus,

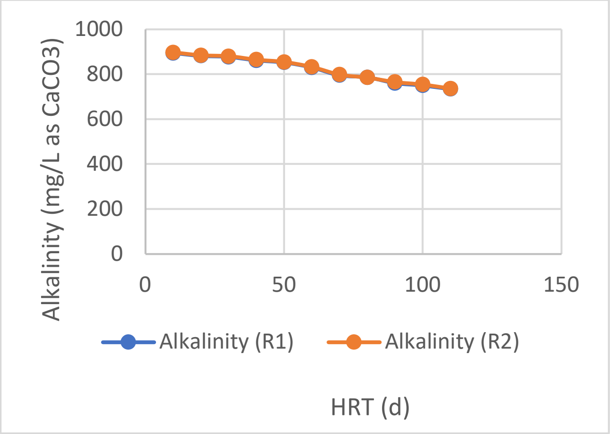


Fig. 7a: Alkalinity variation with HRT in OP I

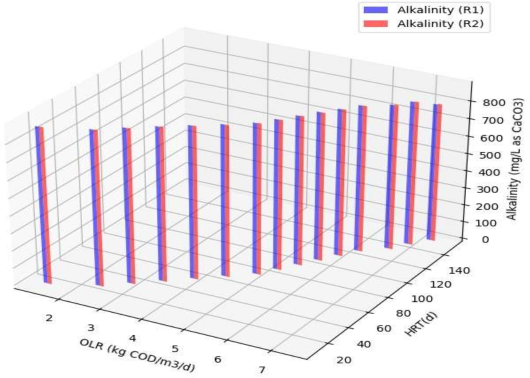


Fig. 7b: Alkalinity variation with varying OLR and HRT in OP II.

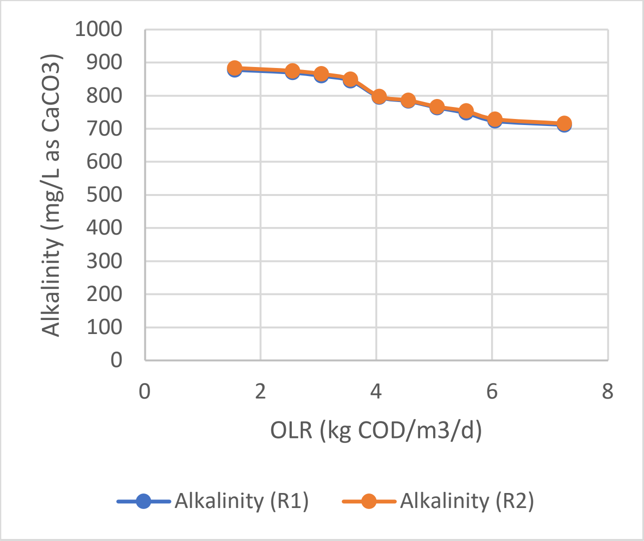


Fig. 7c: Alkalinity variation with OLR in OP III.

the highest BOD reduction in both reactors was noticed at a higher HRT and lower OLR.

COD reduction efficiency: The COD reduction efficiencies of the reactors R1 and R2 in OP I, OP II, and OP III are shown in Fig. 9a, 9b, and 9c, respectively. The highest COD removal efficiencies obtained in reactors R1 and R2 were 92% and 91%, respectively, in OP I at an HRT of 40 d and constant OLR of 3.25 kg COD.m^{-3}.d^{-1}, as shown in Fig. 9a. The COD removal efficiencies of both reactors remained almost stationary after 40 d up to 110 d in OP I. Fig. 9b shows that the highest COD reduction efficiencies achieved in reactors R1 and R2 were 90% and 89%, respectively, in OP II at an OLR of 1.50 kg COD.m^{-3}.d^{-1}and HRT of 10 d. Similarly, in OP III, the highest COD reduction efficiencies attained in reactors R1 and R2 were 78% and 77%, respectively, at an OLR of 1.55 kg COD/m^{3}/d and constant HRT of 5 d as shown in Fig. 9c. In this phase, COD removal efficiencies were decreased as the OLR was enhanced from 1.55 kg COD.m^{-3}.d^{-1}. Thus, the highest COD reduction in both reactors was noticed at a higher HRT and lower OLR.

Lignin reduction efficiency: The lignin reduction efficiencies of the reactors R1 and R2 in OP I, OP II, and OP III are shown in Fig. 10a, 10b, and 10c, respectively. Fig. 10a shows that at an HRT of 40 d and constant OLR of 3.25 kg COD.m-3.d-1, the highest lignin removal efficiencies obtained in reactors R1 and R2 were 84% and 82%, respectively, in OP I; the lignin removal efficiencies of both reactors remained almost stationary after 40 d up to 110 d. Fig. 10b shows that the highest lignin removal efficiencies obtained in reactors R1 and R2 were 82% and

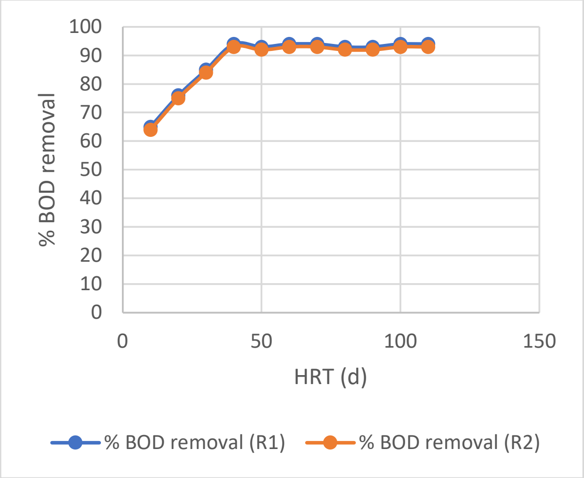


Fig. 8a: Variation of % BOD removal with HRT in OP I.

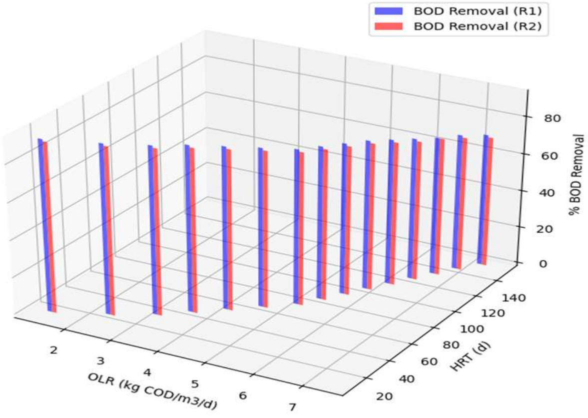


Fig. 8b: Variation of % BOD removal with varying OLR and HRT in OP II.

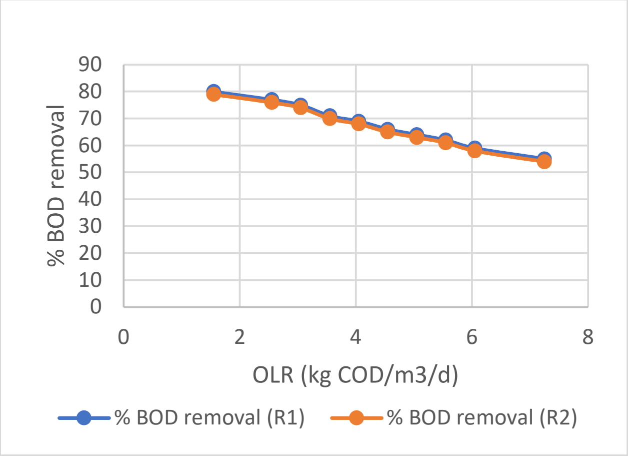


Fig. 8c: Variation of % BOD removal with OLR in OP III.

80%, respectively, in OP II at an OLR of 1.50 kg COD.m-3.d-1 and HRT of 10 d. Similarly, in OP III, the highest lignin removal efficiencies achieved in reactors R1 and R2 were 69% and 67%, respectively, at an OLR of 1.55 kg COD.m-3.d-1 and constant HRT of 5 d as shown in Fig. 10c. In this phase, the lignin removal efficiencies were decreased as the OLR was enhanced from 1.55 kg COD.m-3.d-1. Thus, the highest lignin reduction in both reactors was observed at a higher HRT and lower OLR.

Phenol reduction efficiency: The phenol reduction efficiencies of the reactors R1 and R2 in OP I, OP II, and OP III are shown in Fig. 11a, 11b, and 11c, respectively. As shown in Fig. 11a, the highest phenol removal efficiencies obtained in OP I in reactors R1 and R2 were 82% and 80%, respectively, at an HRT of 40 d and constant OLR of 3.25 kg COD.m^{-3}.d^{-1}; the phenol removal efficiencies of both reactors

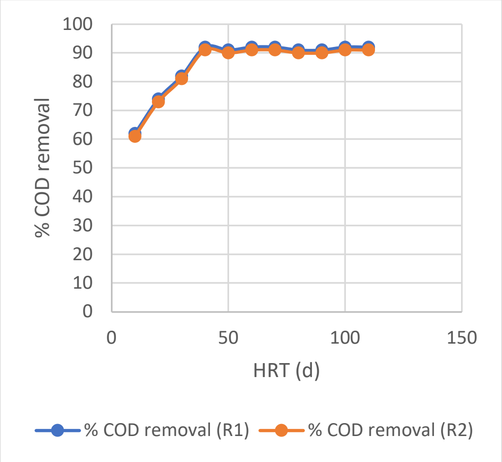


Fig. 9a: Variation of % COD removal with HRT in OP I.

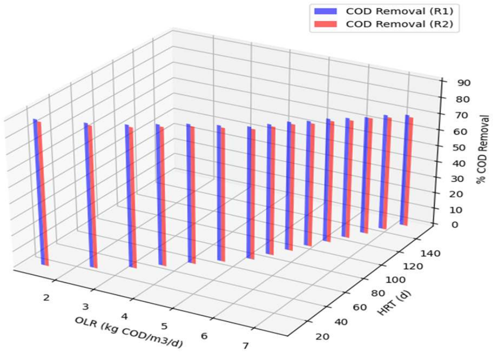


Fig. 9b: Variation of % COD removal with varying OLR and HRT in OP II.

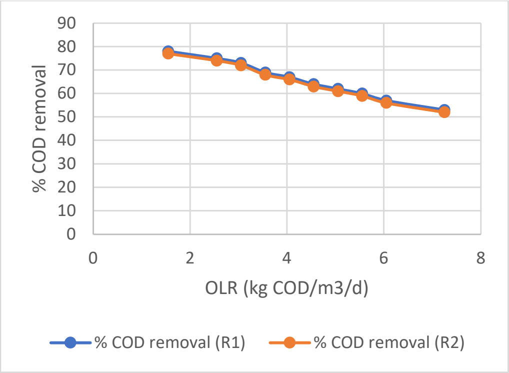


Fig. 9c: Variation of % COD removal with OLR in OP III.

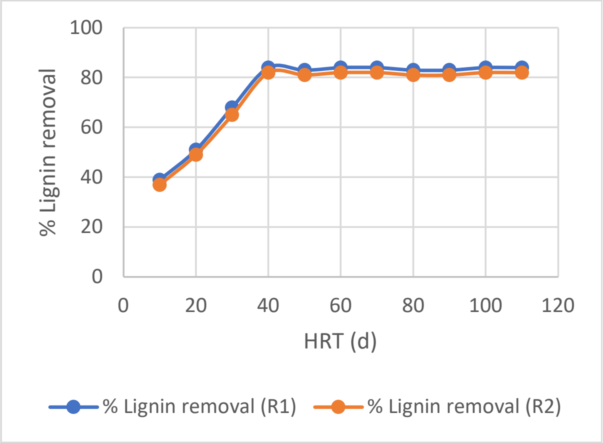


Fig. 10a: Variation of % Lignin removal with HRT in OP I.

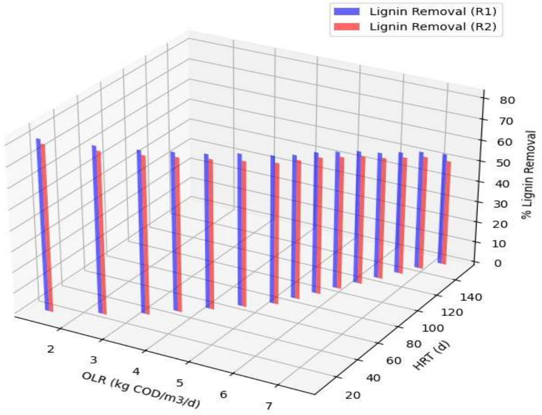


Fig. 10b: Variation of % Lignin removal with varying OLR and HRT in OP II.

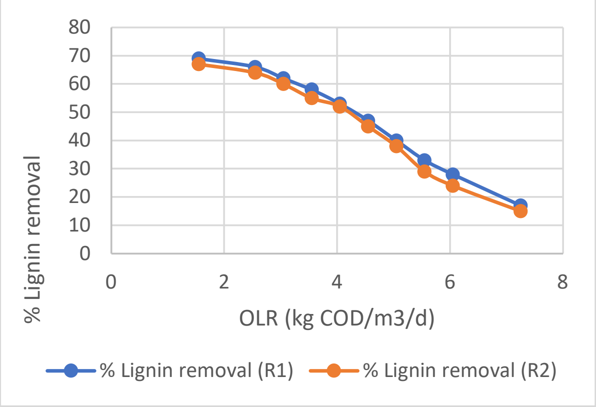


Fig. 10c: Variation of % Lignin removal with OLR in OP III.

remained almost stationary after 40 d up to 110 d. Fig. 11b shows that the highest phenol removal efficiencies obtained in reactors R1 and R2 were 80% and 76%, respectively, in OP II at an OLR of 1.50 kg COD.m^{-3}.d^{-1} and HRT of 10 d. Similarly, in OP III, the highest phenol removal efficiencies achieved in reactors R1 and R2 were 66% and 64%, respectively, at an OLR of 1.55 kg COD.m^{-3}.d^{-1} and constant HRT of 5 d, as shown in Fig. 11c. In this phase, the phenol removal efficiencies were decreased as the OLR was increased from 1.55 kg COD.m^{-3}.d^{-1}. Thus, the highest phenol reduction in both reactors was observed at a higher HRT and lower OLR.

On observing the graphs plotted in Fig. 8, 9, 10, and 11 depicting the BOD, COD, lignin, and phenol removal efficiencies, respectively, in the three operating phases at different OLR and HRT conditions, it can be concluded that the highest pollutant reduction efficiencies of both reactors R1 and R2 occurred at a lower OLR and higher HRT. The pollutant removal efficiency of R1 was only slightly more than that of R2, but the alkali pretreatment of R1 by NaOH was considerably costlier than that of R2 by $Ca(OH)_2$. The highest BOD, COD, lignin, and phenol removal efficiencies of R1 were 94%, 92%, 84%, and 82%, respectively, whereas those of R2 were 93%, 91%, 82%, and 80% respectively. The BOD and COD contents of effluents from reactors R1 and R2 were 223 mg.L^{-1} and 434 mg.L^{-1} as well as 261 mg.L^{-1} and 488 mg.L$^{-1,}$ respectively, at their highest pollutant removal efficiencies. Thus, BOD and COD contents of the effluents from both reactors complied with the effluents discharge standards for safe disposal into public sewers (BOD= 350 mg.L^{-1}, COD- Not defined) as per the Environmental (Protection) Rules, 1986, Govt. of India, New Delhi. Furthermore, the effluents from both reactors may be used for irrigating crops after minor physicochemical treatment like electrocoagulation or adsorption on chitosan (Choudhary et al. 2015, Thirugnanasambandham et al. 2013, Kandagatla et al. 2023) complying with the effluent discharge standards (BOD= 100 mg.L^{-1}, COD= 250 mg.L^{-1}) for irrigation. The significant removal of the two xenobiotic compounds lignin and phenol from RMWW by the two reactors R1 and R2 was feasible due to the presence of lignin and phenol removing bacterial species in the cow dung fed BGPS (inoculum) as well as the bioremediation of the substrate with the white rot fungi (*Phanerochaete chrysosporium*).

Costa et al. (2017) worked on the bioremediation of lignin present in synthetic wastewater as well as industrial paper and pulp mill wastewater (PPMWW) by mycoremediation with white rot fungi viz., *Phanerochaete chrysosporium*

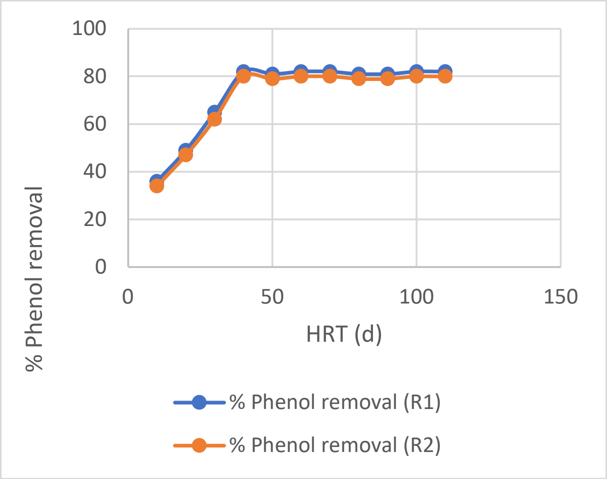


Fig. 11a: Variation of % Phenol removal with HRT in OP I.

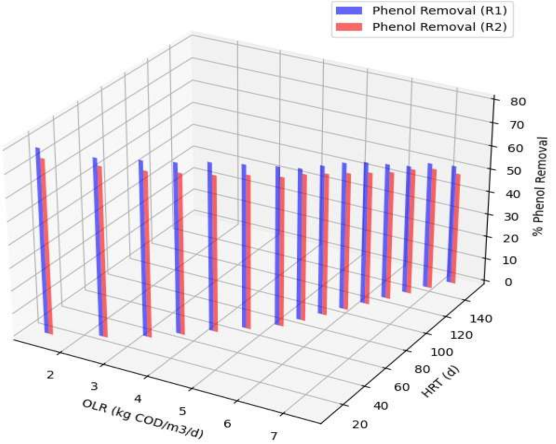


Fig. 11b:Variation of % phenol removal with varying OLR and HRT in OP II.

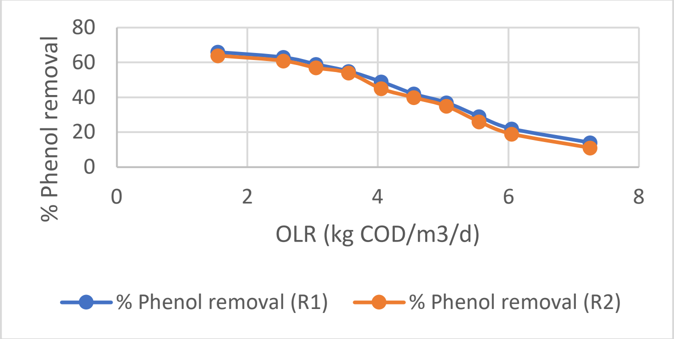


Fig. 11c: Variation of % Phenol removal with OLR in OP III.

and *Bjerkandera adusta*. The researchers concluded that *P. chrysosporium* and *B. adusta* removed 74% and 97% of lignin from the synthetic wastewater, whereas both fungal strains removed 100% of lignin from the PPMWW in 8 to 10 days. Ahmadi et al. (2006) studied the bioremediation of phenol removal from olive mill wastewater (OMWW) with white rot fungi (*Phanerochaete chrysosporium*). The researchers diluted the OMWW with the solution of mineral salt blended with ammonium sulfate, glucose, and yeast. The fungi thrived in the diluted OMWW and removed 90% of phenol from the OMWW.

Kumar et al. (2022) studied the biodegradation of paper and pulp mill wastewater (PPMWW) to remove lignin using the bacterial strain *Bacillus* sp. The researchers concluded that *Bacillus* sp. removed 89% of lignin and 40% of color at 1000 $mg.L^{-1}$ lignin in an HRT of 3 days. Singh et al. (2022) studied the biodegradation of paper and pulp mill sludge (PPMS) to remove lignin by the bacterial strain *Bacillus* sp. The researchers found that *Bacillus* sp. removed 84% of lignin in an HRT of 14 days. Haq and Kalamdhad (2023) studied the bioremediation of paper and pulp mill wastewater (PPMWW) by the bacterial strain *Pseudomonas* sp. The researchers found that the *Pseudomonas* sp. removed 65.6% of lignin, 85.7% of color, and 98.4% of phenol from the wastewater in an HRT of 6 days. De Angelis et al. (2013) worked on lignin removal from industrial wastewater by *Enterobacter* sp. in anaerobic conditions. The researchers found that the microbial strain removed 56% of lignin from the wastewater in an HRT of 48 h. Yadav et al. (2022) studied the microbial degradation of lignin and phenol. The researchers found that the bacterial strains *Bacillus* sp., *Pseudomonas* sp., *Actinomycetes* sp. and *Streptomycetes* sp. can biodegrade lignin and dihydroxyl phenol from industrial wastewater.

Reddy et al. (2017) studied the in-situ biodegradation of phenol from phenol-laden contaminated wastewater using *Bacillus* sp. The researchers found that the bacterial sp. removed 84% of phenol from the contaminated wastewater in an HRT of 6 days. Ke et al. (2018) carried out an investigation into the bioremediation of phenol from phenol-laden synthetic wastewater using *Bacillus* sp. The researchers concluded that the microbial sp. removed 87.2% and 100% of phenol in an HRT of 12 h and 24 h, respectively, from the synthetic wastewater. Diksha et al. 2023 studied the phenol-rich sewage by *Bacillus* sp. at 1250 $mg.L^{-1}$ phenol. The researchers concluded that the bacterium removed 83.9% of phenol from the sewage in an HRT of 13 days. Song et al. (2009) studied the biodegradation of phenol and Cr (VI) by *Pseudomonas* sp. in a reactor in the mineral liquid medium. They found that the microbial strain removed 70.5% of phenol and 83.2% of Cr (VI) from the liquid medium. Mahgoub et al. (2023) studied the biodegradation of phenolic wastewater by *Pseudomonas* sp. in a mineral salt medium. They found that the bacterium removed 74.68% of phenol at 1.0 $g.L^{-1}$ in an HRT of 3 days and tolerated high phenol contents up to 2.0 $g.L^{-1}$. Stoilova et al. 2006 investigated the biodegradation of high amounts of phenol in a synthetic phenol solution with the help of the filamentous fungal strain *Aspergillus* sp. The findings revealed that 85% of phenol was degraded in an HRT of 6 days. El-Din 2023 studied the biodegradation of phenol from a synthetic phenol solution by the marine fungal strain *Aspergillus* sp. The study revealed that 88% of phenol was reduced from the synthetic solution in an HRT of 168 h at 31°C temperature.

Sharma and Singh (2015) carried out a morpho-biochemical analysis of the cow dung. The test results revealed that the bacteria present in cow dung belonged to *Bacillus* sp., *Pseudomonas* sp., *Salmonella* sp., etc. The biochemical tests to characterize the microorganisms present in cow dung aimed at bio-remediating the potent xenobiotic compound benzene were carried out by Godambe and Fulekar (2016). The researchers selected 10 well-isolated bacterial colonies for testing. The test results revealed that the microorganisms present in cow dung belonged to *Basilus* sp., *Pseudomonas* sp., *Enterobacter* sp., etc. A microbial investigation to determine the bacterial diversity existing in cow dung was conducted by Munshi et al. (2018). The results revealed that the microorganisms present in cow dung predominantly belonged to *Basilus* sp., *Pseudomonas* sp., *Salmonella* sp., etc. The microorganisms present in cow dung were studied by Devi et al. (2023). The researcher found that the cow dung inhabited plentiful microbial diversity comprising enormous bacterial, fungal, and protozoan species such as *Bacillus* sp., *Pseudomonas* sp. and *Aspergillus* sp. etc.

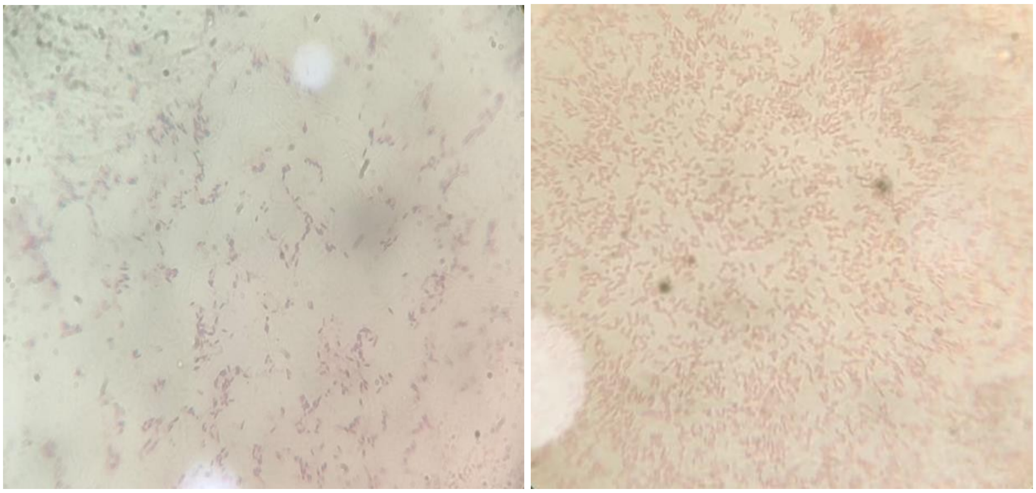
Microbial Analysis of the Anaerobic Sludge from the Lab-scale Bioreactors

The microbial analysis of the anaerobic sludge from the lab-scale bioreactors was performed in the Department of Biotechnology, Pandit Ravishankar Shukla University, Raipur, Chhattisgarh, India. The analytical reports revealed that the digestate comprised the bacterial strains *Bacillus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Actinomycetes* sp. and *Streptomycetes* sp. as well as the fungal strain *Aspergillus* sp., which confirms the significant removal of lignin and phenol. The evidence of microbial species present in anaerobic sludge is shown in Fig. 12, 13 and 14.

Analyses of Biogas Generation and Biomethane Yield in Three Different Operating Phases

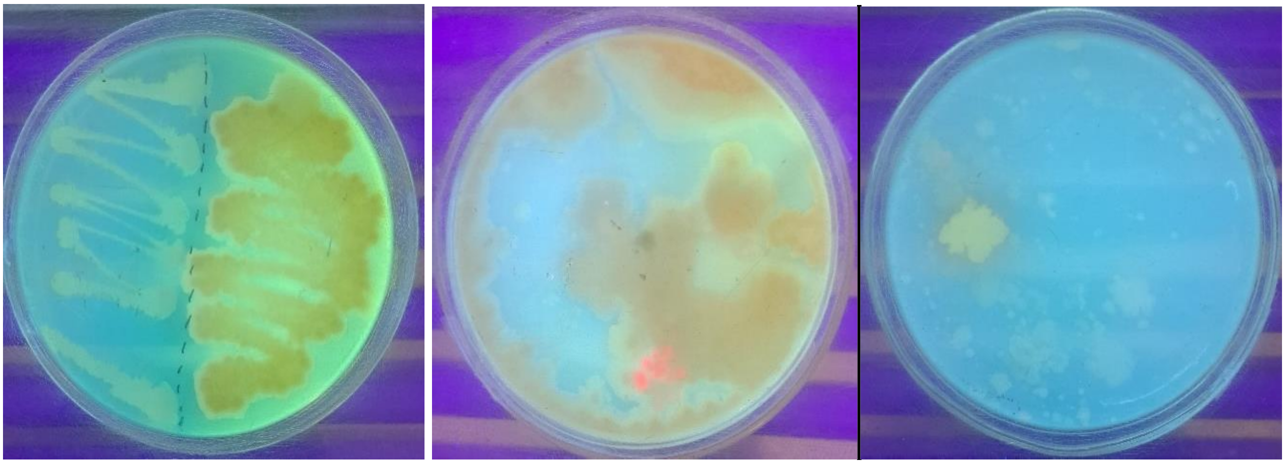
The biogas generations from anaerobic digestion of RMWW

***Bacillus* sp.**



Gram +ve *Bacillus* sp. Gram -ve *Bacillus* sp.

***Pseudomonas* sp.**



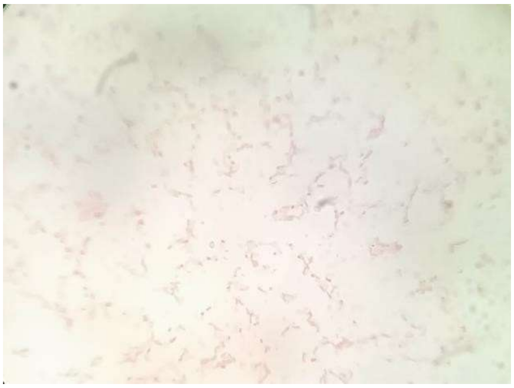
Pseudomonas fluorescens sp. Culture on King's medium emits fluorescence under UV light.

Fig. 12: Evidence of *Bacillus* sp. and *Pseudomonas* sp.

in ASCR- R1 and R2 under different OLR and HRT conditions are given in Tables 5a, 5b, 5c, 6a, 6b, and 6c, respectively. After 16 days of the reactors' stabilization and 4 days of the arrival of uniform and steady biogas generation from the reactors after initial fluctuations, the biogas produced was monitored daily. The cumulative sum of the biogas generated from both reactors was separately calculated every 10 days, shown in the tables mentioned earlier against each HRT. In Operating Phase I (OP I), the HRT was varied from 10 d to 110 d, and OLR was kept constant at 3.25 kg COD.$m^{-3}.d^{-1}$; the highest biogas generation was visualized after an HRT of 40 d. After hydraulic retention of 40 d, the biogas generated from both reactors were nearly stationary throughout 110 d, which is illustrated by the above-cited tables. In OP II, the HRT was changed from 10 d to 150 d, and q the OLR was changed from 1.50 to 7.32 kg COD.$m^{-3}.d^{-1}$; the highest generation of biogas was visualized at an HRT of 110 d and an OLR of 6.25 kg COD.$m^{-3}.d^{-1}$. In OP III, the OLR was changed from 1.55 to 7.25 kg COD.$m^{-3}.d^{-1}$, and HRT was kept constant at 5 d for each loading rate; the highest biogas

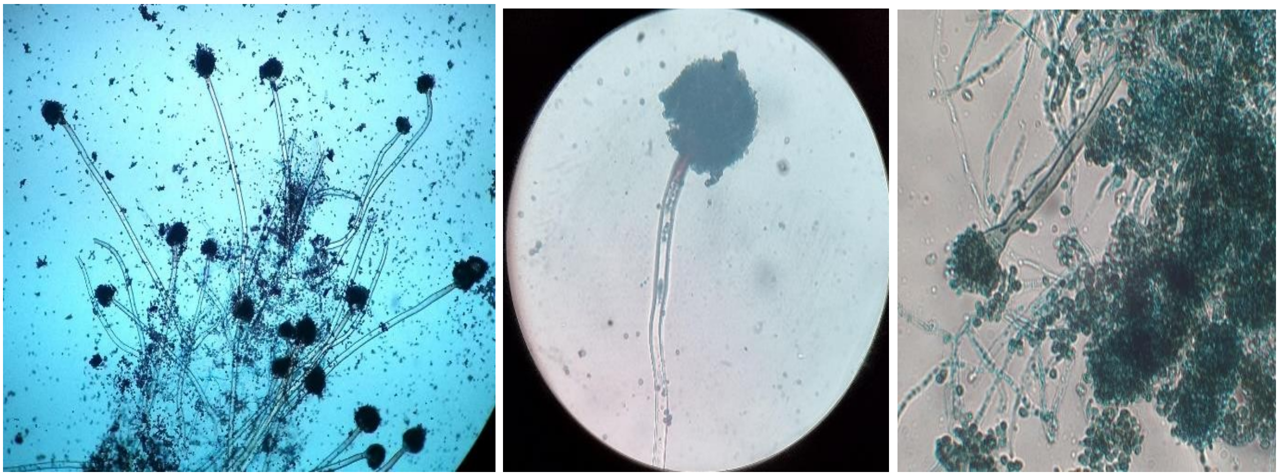
***Enterobacter* sp.**

Gram-negative, rod-shaped. Culture grows under facultative anaerobic conditions.



Gram -ve *Bacillus* sp.

***Aspergillus* sp.**



Aspergillus niger

Aspergillus sp.

Fig. 13: Evidence of *Enterobacter* sp. and *Aspergillus* sp.

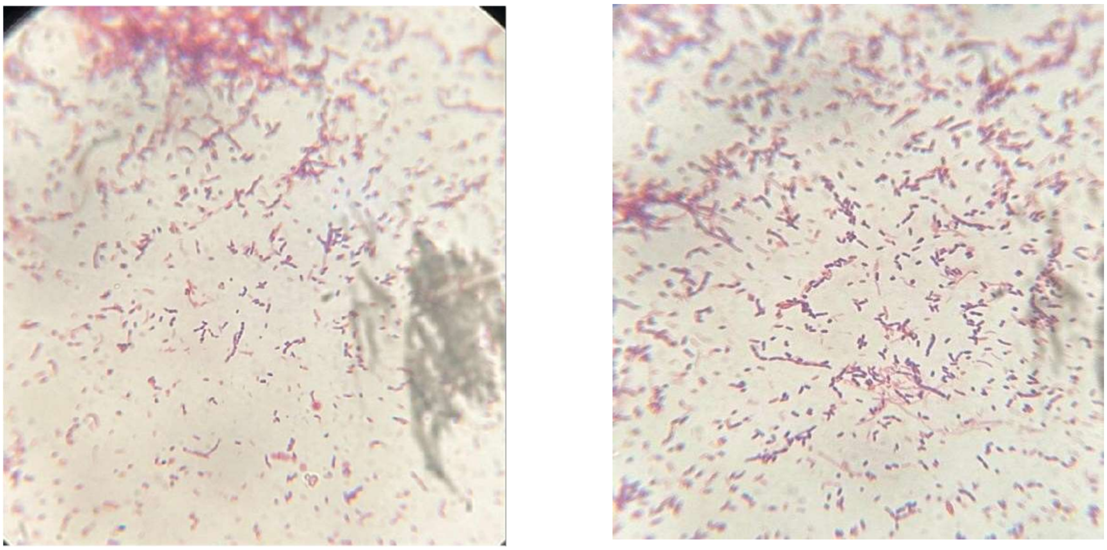
generation was visualized at an OLR of 7.25 kg $COD.m^{-3}.d^{-1}$. These results manifested that the maximum biogas generation from reactors- R1 and R2 was obtained at an enhanced OLR and HRT. The highest biogas generation from both reactors in different operating phases is shown in Fig. 15.

Biomethane yield is another paramount parameter for assessing the bioreactor's performance. The maximum biomethane yield in operating phases I, II, and III from the anaerobic semi-continuous reactors- R1 and R2 are shown in Fig. 16. The biomethane yield ranged from 0.002-0.005 $L.g^{-1}$ COD in both reactors throughout the operation process. The results indicate that the biomethane yield depends on OLR conditions. A low OLR value ushered in low organic contents in the substrate of the reactors, which ultimately ushered in low biomethane yield. On the contrary, too high OLR resulted in an improper food-to-microorganisms (F/M)

***Actinomycetes* sp.**

Gram +ve *Bacillus* sp.

***Streptomyces* sp.**



Streptomyces sp. Gram +ve *Bacillus* sp.

Fig. 14: Evidence of *Actinomycetes* sp. and *Streptomyces* sp.

ratio, which also resulted in a low biomethane yield. Thus, an optimum OLR is suitable for attaining higher biomethane yield. The maximum biomethane yield (0.005 $L.g^{-1}$ COD) achieved in OP II in both reactors was the highest among all three operating phases, as shown in Fig. 16.

Analyses of the Digestates from Bioreactors- R1 and R2

After the completion of the biological treatment of the RMWW in ASCR- R1 and R2 in the three operating phases I, II, and III, the digestates from both reactors were evacuated.

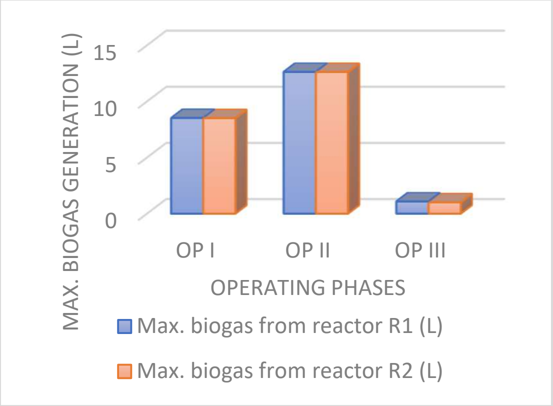


Fig. 15: Maximum biogas generation in operating phases I, II, and III.

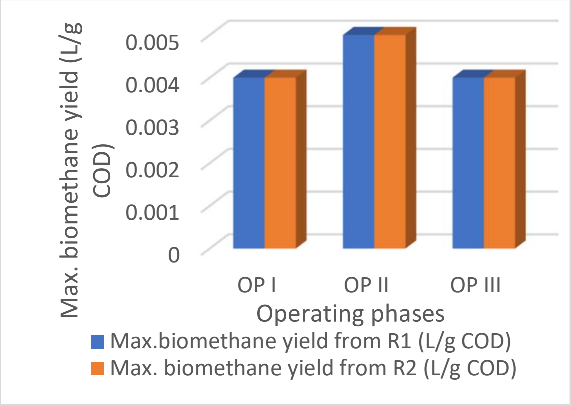


Fig. 16: Maximum biomethane yield in operating phases I, II, and III.

Small quantities of the digestates from both reactors were taken out for analysis. The analyte digestates were dried in the lab oven at 103°C-105°C so that the mechanically occluded water present in the digestates was almost driven out. The dried analyte digestates were properly desiccated in the desiccator. Eventually, the dried and desiccated digestates from reactors R1 and R2, as shown in Fig. 17 and 18, were analyzed by Scanning Electron Microscope with Energy-Dispersive X-ray Spectroscope (SEM/EDX) in the Department of Metallurgical and Materials Engineering (SEM Lab), NIT Raipur, Chhattisgarh, India. As per the SEM/EDX reports, digestate from the bioreactor R1 (BRD-1) and the digestate from the bioreactor R2 (BRD-2) both contain the macronutrients viz., N, P, K, Ca, Mg, Al, Na, and micronutrients viz., Fe, Zn, Cu, Mn, Cl, C, O, etc., essential for the plant growth. The analytical reports of SEM/EDX of BRD-1 and BRD-2 are shown in Fig. 19a, 19b, and 20a, 20b, respectively.

The digestate samples BRD-1 and BRD-2 were also sent to the Department of Soil Science and Agricultural Chemistry, Indira Gandhi Krishi Vishwavidyalaya (IGKVV) Raipur, Chhattisgarh for quantitative analyses of the essential macro and micronutrients present in the samples. As per the chemical analysis report of IGKVV Raipur, both BRD-

Table 7: Analytical report of digestates from bioreactors- R1 & R2 i.e., BRD-1 & BRD-2.

Essential plant nutrients (macro/micro)	Nitrogen (N)	Phosphorus (P)	Potassium (K)	Zinc (Zn)	Copper (Cu)	Manganese (Mn)	Iron (Fe)
BRD-1 [in g.kg^{-1} i.e., ppm]	14,500	2,600	12,900	23	6	47	104
BRD-2 [in g.kg^{-1} i.e., ppm)	14,900	2,300	11,400	19	7	43	107

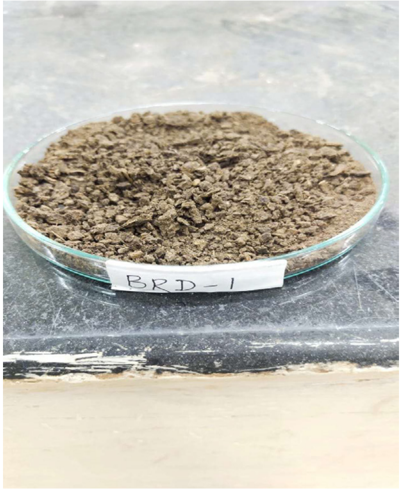


Fig. 17: Oven-dried and crushed BRD-1.

Fig. 18: Oven-dried and crushed BRD- 2.

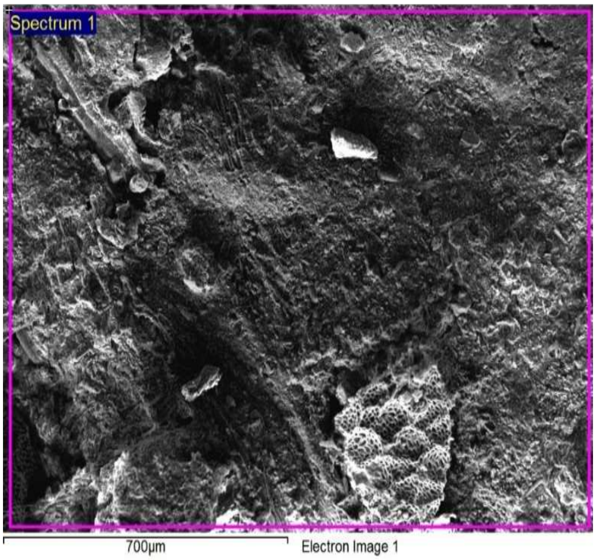


Fig. 19a: SEM/EDX image of BRD-1.

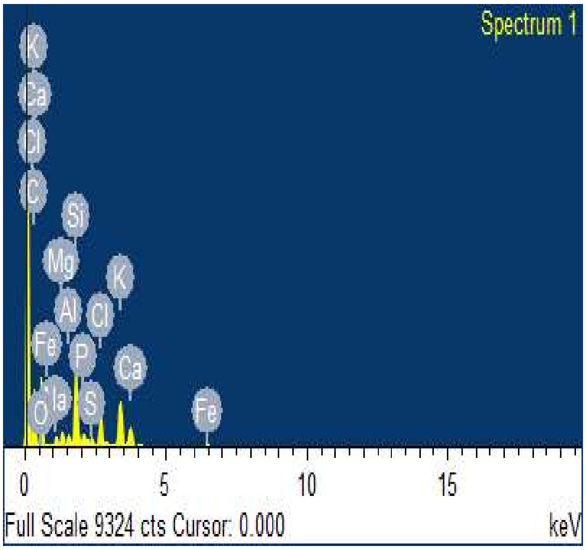


Fig. 19b: SEM/EDX spectrum of BRD-1.

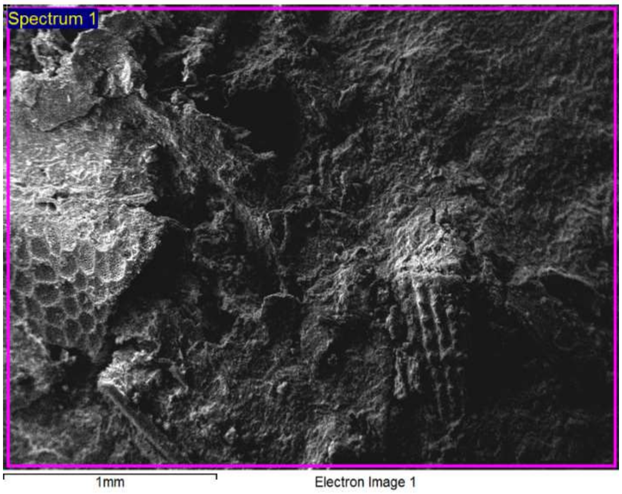


Fig. 20a: SEM/EDX image of BRD-2.

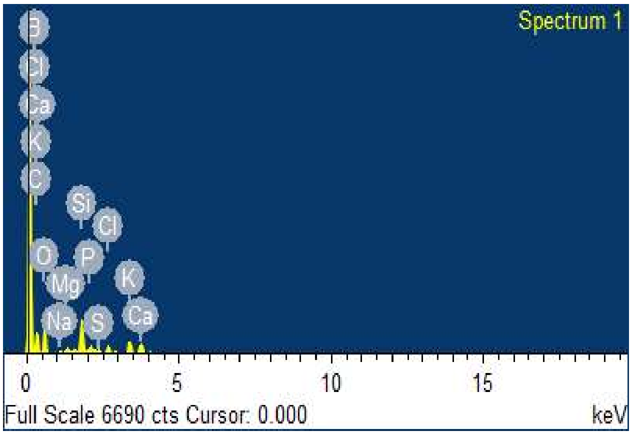


Fig. 20b: SEM/EDX spectrum of BRD-2.

1 and BRD-2 contain appropriate amounts of macro and micronutrients essential for plant growth, which are shown in Table 7. As a result, digestates of the reactors dealing with the biological anaerobic treatment of the rice mill wastewater may be used as biofertilizers for crops and as soil conditioners since they contain oxygen also according to the SEM/EDX reports.

CONCLUSIONS

The study proposed biological anaerobic treatment of rice mill wastewater, which significantly removed the pollutants and produced biomethane (CH_4). The highest pollutant removal efficiencies were observed in both reactors at an HRT of 40 d and onward in OP I. The highest BOD, COD, lignin, and phenol removal percentages achieved in reactors R1 and R2 were 94%, 92%, 84%, 82%, 93%, 91%, 82%, and 80%, respectively. The highest biogas generation was achieved at an HRT of 110 d and OLR of 6.25 kg $COD.m^{-3}.d^{-1}$ in both reactors in OP I. The highest biomethane yield was achieved in both reactors at an OLR of 3.25 kg $COD.m^{-3}.d^{-1}$ and an HRT of 30 d in OP II. The alkali pretreatment of RMWW by $Ca(OH)_2$ in bioreactor R2 gave almost a similar pollutant removal efficiency as NaOH pretreatment. Hence, alkali pretreatment of RMWW by $Ca(OH)_2$ may be recommended in rice mills because it is cheaper than NaOH. The pollutant removal, biogas yield, and biomethane yield were analyzed under varying conditions of HRT and OLR. A long HRT and low OLR conditions provided the maximum pollutant removal efficiency and biomethane yield. The reactor digestates were rich in macro and micronutrients essential for plant growth.

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