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Original Research

Performance Evaluation of a Coconut Coir Immobilized Biphasic Anaerobic Reactor for High-Strength Pharmaceutical Wastewater Treatment: Identification of Dominant Microbial Isolates

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Abstract: Pharmaceutical manufacturing industries generate wastewater containing high concentrations of organic matter and several inhibitory compounds that can adversely affect conventional biological treatment processes. In the present study, the performance of a laboratory-scale biphasic anaerobic reactor was investigated for the treatment of high-strength pharmaceutical wastewater under mesophilic conditions. The system consisted of an acidogenic suspended-growth reactor followed by a methanogenic attached-growth reactor packed with coconut coir as a natural support medium to enhance microbial immobilization and biomass retention.

The reactor was operated at organic loading rates ranging from 0.5 to 3.558 kg COD/m³·d, while the hydraulic retention times were maintained at 24 h and 72 h in the acidogenic and methanogenic reactors, respectively. The acidogenic reactor maintained a pH range between 4.65 and 5.9, whereas the methanogenic reactor operated within a stable pH range of 7.11–8.38, indicating effective phase separation. Maximum COD removal efficiency of 87.54% was observed at lower organic loading rates, while a reduction to 64.68% occurred at higher loading conditions due to temporary accumulation of volatile fatty acids.

Microbial identification revealed the dominance of *Microbacterium enclense* in the acidogenic phase and *Methanococcus aeolicus* in the methanogenic phase. The results indicate that coconut coir provides a suitable natural support medium for microbial attachment and improves the stability of biphasic anaerobic treatment systems.

Keywords: Pharmaceutical wastewater; Biphasic anaerobic reactor; Coconut coir; Organic loading rate; COD removal; Reactor stability.

1. INTRODUCTION

Pharmaceutical manufacturing processes generate complex wastewater containing active pharmaceutical ingredients, solvents, intermediates, and auxiliary chemicals used during production. These effluents often exhibit high values of Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), suspended solids, and recalcitrant organic compounds (Verlicchi et al. 2012; Zhang et al. 2014). If discharged without adequate treatment, such wastewater can adversely affect aquatic ecosystems and may contribute to the development of antibiotic-resistant microorganisms (Kümmerer 2009; Ahmed et al. 2020).

Conventional aerobic treatment systems are widely used for industrial wastewater treatment; however, their performance may be limited when treating high-strength pharmaceutical effluents due to toxicity, shock loading, and the presence of inhibitory compounds. Anaerobic treatment processes offer several advantages over aerobic systems, including lower sludge production, reduced energy requirements, and the possibility of energy recovery in the form of methane-rich biogas (Lettinga and Hulshoff Pol 1991; McCarty 2001).

Two-stage anaerobic digestion systems have been increasingly investigated because they allow hydrolysis and acidogenesis to occur separately from methanogenesis. This separation enables improved control of environmental conditions required for different microbial groups and can enhance the stability of the treatment process (Demirel and Yenigun 2002). Nevertheless, biomass washout and insufficient microbial retention remain major operational challenges when reactors are subjected to high organic loading rates.

In recent years, the use of natural carrier materials for biomass immobilization has gained attention due to their low cost and environmental compatibility. Coconut coir, a lignocellulosic by-product obtained from coconut husk, possesses a porous fibrous structure that provides suitable surfaces for microbial attachment and biofilm development (Li et al. 2023). Such characteristics make it a promising support material for improving biomass retention in anaerobic reactors.

The present study therefore investigates the performance of a coconut coir–packed biphasic anaerobic reactor for the treatment of high-strength pharmaceutical wastewater. In addition to reactor performance, the study also examines the microbial communities involved in the acidogenic and methanogenic phases and their relationship to process stability under varying organic loading conditions.

2. MATERIALS AND METHODS

2.1. Bio-carrier Material

Coconut coir was used as a natural bio-carrier in the methanogenic reactor due to its high porosity, rough surface texture, and biodegradability. The coir fibers ranged from 6–30 cm in length with moisture content less than 5% and impurity content below 8%. Before use, the coir fibers were washed with tap water to remove dust and impurities, oven-dried at 60 °C for 24 h, and sterilized to prevent contamination.

2.2. Biphasic Anaerobic Reactor Configuration

A laboratory-scale biphasic anaerobic reactor fabricated from plexiglass with a total working volume of 36.92 L was used in this study. The reactor consisted of an acidogenic reactor (AR) with a working volume of 6.15 L operated as a suspended growth system, followed by a methanogenic reactor (MR) with a working volume of 30.77 L operated as an attached growth system packed with coconut coir. The AR and MR were connected in series with independent gas collection arrangements. Wastewater was fed to the system using a peristaltic pump (Model PP-30 EX), and the influent and effluent pipelines were maintained at a diameter of 1 cm. The schematic configuration of the biphasic anaerobic reactor is illustrated in Fig. 1.

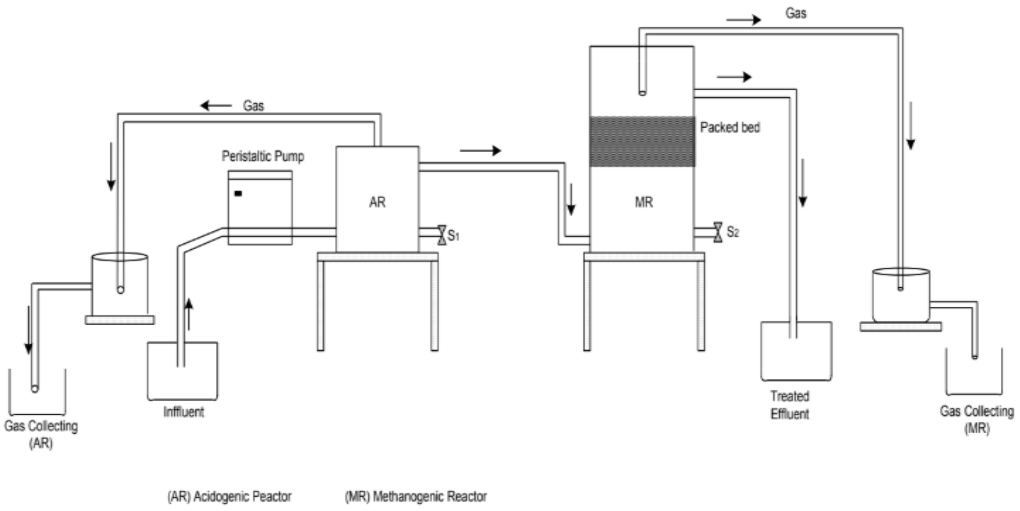


Fig. 1: Schematic representation of biphasic anaerobic reactor.

The reactor was operated under ambient mesophilic conditions (28–32 °C). During reactor start-up, the organic loading rate was gradually increased to allow microbial populations to acclimatize to the wastewater. The Hydraulic Retention Time (HRT) in the acidogenic and methanogenic reactors was maintained at 24 h and 72 h, respectively, to ensure effective phase separation and stable operation. Wastewater was fed using a peristaltic pump (Model PP-30 EX). The influent and effluent pipe diameter was maintained at 1 cm.

2.3. Sample Collection and Characterization

Pharmaceutical wastewater and seed sludge were collected from M/s Life Care Formulations, Puducherry, India. The seed sludge concentration was maintained at 4.5 g/L throughout the study. Physico-chemical parameters of the wastewater were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA 2017). Arsenic was analyzed by standard APHA 2027 procedures using Atomic Absorption

Spectrophotometry (AAS). The influent pharmaceutical wastewater exhibited high organic strength and inhibitory characteristics as summarized in Table 1. The operating conditions maintained in both reactor phases are presented in Table 2. The values were compared with Central Pollution Control Board (CPCB) discharge standards for industrial effluents.

Table 1: Physico-chemical characteristics of influent pharmaceutical wastewater.

Parameter	Unit	Value
pH	----	4.57
COD (mg/L)	mg/L	16833
BOD_5 (mg/L)	mg/L	7623
TSS (mg/L)	mg/L	6967
TDS (mg/L)	mg/L	667
Oil & Grease (mg/L)	mg/L	58
Phosphate (mg/L)	mg/L	145
Arsenic (mg/L)	mg/L	0.651
Lead (mg/L)	mg/L	0.26
Chromium (mg/L)	mg/L	0.38

Table 2: Operating parameters of the biphasic anaerobic reactor.

Parameter	Acidogenic Reactor	Methanogenic Reactor
Working Volume	6.15 L	30.77 L
HRT	24 h	72 h
Temperature	28-32^{0}C	28-32^{0}C
OLR	0.5–3.558 kg COD/m³·d	0.5–3.558 kg COD/m³·d
Bio-carrier	None	Coconut Coir

3. Microbial Isolation and Identification

Samples were collected at different elapsed operating times within each loading cycle. The x-axis values indicate sampling intervals and not reactor HRT.

3.1. Reactor Treatment Performance

Acidogenic effluent samples were collected from the acidogenic reactor, serially diluted, and plated on nutrient agar. The plates were incubated at 37 °C for 24–48 h under anaerobic conditions using an anaerobic jar. Dominant

colonies were isolated and repeatedly sub-cultured to obtain pure cultures. Morphological characteristics and Gram staining were performed prior to molecular identification.

3.2. Genomic DNA Extraction, PCR Amplification and Phylogenetic Analysis

Genomic DNA was extracted from the pure cultures using a QIAGEN DNA isolation kit following the manufacturer's protocol. The quality of extracted DNA was confirmed by agarose gel electrophoresis. The 16S rRNA gene was amplified using universal bacterial primers 27F (5'-AGAGTTTGATTCCTGGCTCAG-3') and 1522R (5'-AAGGAGGTGAATCCANCCRCA-3'). The 16S rRNA sequencing approach is widely used for bacterial identification and phylogenetic classification (Lane 1991)

PCR amplification was carried out with an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 56 °C for 45 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR products were analyzed on 1% agarose gel and sequenced using an ABI PRISM 3730 genetic analyzer.

The obtained sequences were compared with reference sequences available in the NCBI database using BLASTN. Sequence similarity searches were performed using the BLAST algorithm (Altschul et al. 1990). Multiple sequence alignment was performed using CLUSTAL W, and phylogenetic trees were constructed using MEGA 4 software with 1000 bootstrap replications. Phylogenetic relationships were inferred using the MEGA software platform (Tamura et al. 2007).

3.3. Isolation of Methanogenic Bacteria

A 1 ml portion of methanogenic effluent was subjected to serial dilution, with the 6th and 7th dilutions subsequently inoculated onto sterilized agar plates. After an incubation period of 24-48 hours in a bacteriological incubator, the sample was assessed for bacterial growth. Enumeration of bacterial colonies was conducted, and the prevalent culture was then spread onto a new, sterile nutrient agar plate. Molecular identification of the dominant culture was accomplished through the sequencing of 16S rRNA genes.

4. RESULTS AND DISCUSSIONS

The influent wastewater contained arsenic at 0.651 mg/L (Table 1). This concentration may exert inhibitory effects on anaerobic microorganisms. However, stable reactor performance suggests microbial acclimatization and possible metal adsorption/precipitation within sludge biomass. Further studies on dissolved arsenic speciation and removal are recommended.

4.1. Acidogenic Microbial Community

The dominant microbial species identified in both reactor phases are listed in Table 3. The dominant bacterial isolate from the acidogenic reactor was identified as *Microbacterium enclense*, a Gram-positive, non-motile, non-sporulating rod-shaped bacterium (Zhang et al. 2021).

Table 3: Dominant microbial species identified in biphasic reactor

Reactor Phase	Microorganism	Functional Role
Acidogenic	Microbacterium enclense	Hydrolysis
Methanogenic	Methanococcus aeolicus	Methanogenesis

SEM analysis of acidogenic biomass is presented in Fig. 2. SEM images revealed smooth-surfaced rod-shaped cells, indicating healthy and active biomass. Colonies on nutrient agar were greyish-white and non-pigmented.

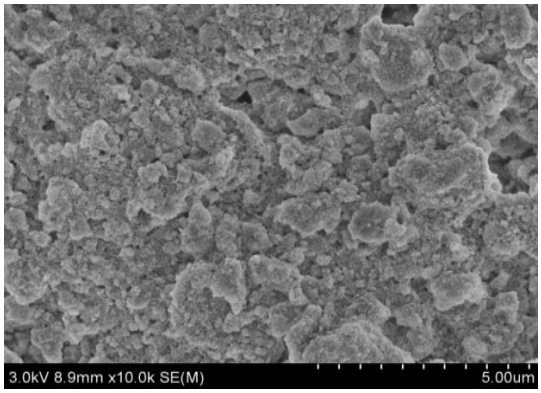


Fig. 2: SEM image of acidogenic effluent.

The detection of Microbacterium species indicates active hydrolytic and acidogenic processes within the reactor, as these bacteria are known to participate in the breakdown of complex organic compounds into simpler volatile fatty acids. Microbacterium species have been reported to participate in hydrolytic degradation of complex organic substrates in anaerobic environments (Zhang et al. 2021). This is particularly important in the treatment of

high-strength pharmaceutical wastewater, where rapid conversion of complex substrates is required to prevent inhibition of downstream methanogens.

4.2. Methanogenic Microbial Community

The methanogenic reactor predominantly contained *Methanococcus -like archaeal isolate*, a Gram-negative, non-sporulating archaeon with coccoid morphology. SEM analysis of Methanogenic biomass is presented in Fig. 3. SEM analysis showed a clustered spherical cell typical of methanogenic archaea. Although *Methanococcus* species are commonly reported in marine environments, similar strains have been identified in saline and high-strength industrial wastewaters, indicating their adaptive capability under stressed and high-strength conditions. Methanococcus species are hydrogenotrophic methanogens commonly detected in saline and high-strength environments (Garcia et al. 2000).

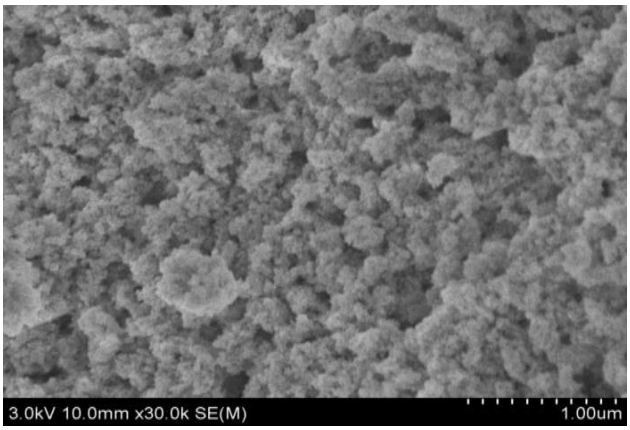


Fig. 3: SEM Image of Methanogenic effluent

The dominance of *Methanococcus aeolicus* confirms effective methanogenesis in the attached growth system. The fibrous morphology of coconut coir provided a suitable surface for microbial attachment and biofilm formation, enhancing biomass retention and stability of the methanogenic phase.

4.3. Role of Coconut Coir as Bio-carrier

The incorporation of coconut coir as an immobilization medium contributed to biomass retention and stable attached growth operation. The irregular fibrous network and intrinsic porosity of the material provided a substantial surface area for biofilm colonization. This structural property helped reduce biomass washout and enhanced

the operational stability of the reactor under fluctuating organic loading conditions. The findings support the feasibility of utilizing natural lignocellulose carriers as sustainable alternatives to synthetic packing materials in anaerobic treatment systems.

4.4. Reactor Stability and Process Performance

The operational stability of the biphasic anaerobic reactor was evaluated through pH variation, volatile fatty acid (VFA) concentration, and volatile suspended solids (VSS) profile in both acidogenic and methanogenic phases and the range of parameter values are presented in Table 4.

Table 4: Performance characteristics of Acidogenic and Methanogenic reactors under varying organic loading rates

Reactor	OLR (kg COD m^{-3} d^{-1})	Influent COD (mg L^{-1})	Effluent COD (mg L^{-1})	COD Removal (%)	pH	VSS (mg L^{-1})	VFA (mg L^{-1})
Acidogenic Reactor (AR)	0.811 – 6.549	1858	1112 – 1656	20.07 – 27.39	5.65 – 5.90	509 – 747	2128 – 2223
	1.492 – 7.611	2864	1888 – 2632	20.41 – 25.73	5.55 – 5.79	849 – 1240	2723 – 3589
	1.933 – 12.64	4051	2920 – 3456	20.03 – 24.73	5.48 – 5.73	1277 – 1474	3589 – 4723
	2.304 – 17.043	5180	5016 – 5472	18.05 – 22.81	5.12 – 5.65	1820 – 2340	4312 – 5896
	2.947 – 21.140	6786	5368 – 5632	15.78 – 21.60	4.68 – 5.35	2207 – 2450	5045 – 6456
Methanogenic Reactor (MR)	0.112 – 1.046	1412	248 – 1416	70.52 – 77.76	8.11 – 8.21	378 – 579	454 – 546
	0.221 – 1.210	2198	270 – 504	68.80 – 77.00	7.98 – 8.16	743 – 899	534 – 632
	0.290 – 2.021	3174	848 – 952	70.25 – 75.46	7.60 – 7.92	934 – 1140	635 – 700
	0.355 – 2.042	4291	1183 – 1273	67.20 – 71.94	7.18 – 7.56	1487 – 1750	680 – 786
	0.461 – 2.637	5526	1608 – 1984	64.68 – 70.53	7.11 – 7.42	1811 – 1971	754 – 884

4.4.1. pH Variation in Acidogenic and Methanogenic Reactors

The distinct pH ranges in the two reactors confirm effective phase separation as depicted in Fig. 4 and Fig. 5. Acidic conditions in the first stage enhanced hydrolysis and fermentation, while neutral to slightly alkaline conditions in the second supported methanogenesis. Stable pH in the methanogenic reactor indicates good buffering and efficient conversion of intermediate acids, even at higher loading.

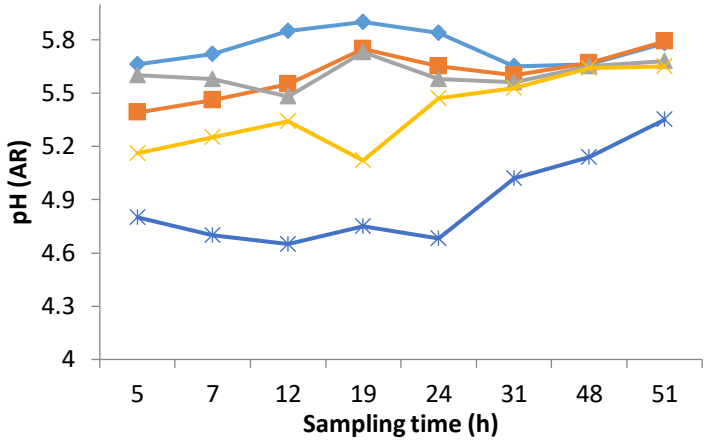


Fig. 4: Effect of sampling time (h) on pH in Acidogenic reactor (AR)

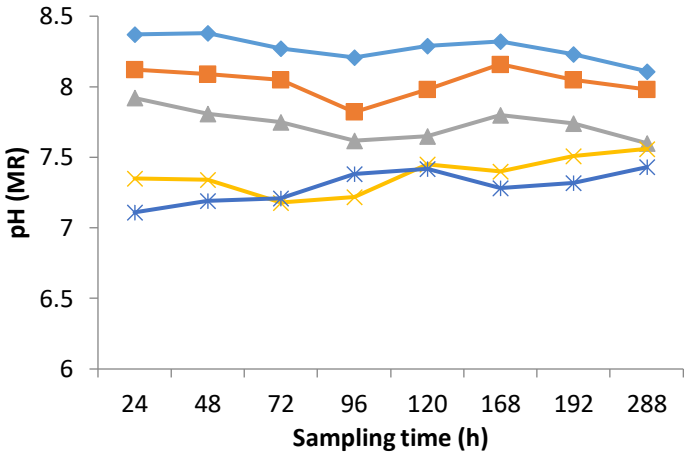


Fig. 5: Effect of sampling time (h) on pH in Methanogenic reactor (MR)

4.4.2. Volatile Fatty Acid Profile

Elevated VFA concentrations detected in the acidogenic reactor reflect active substrate fermentation as shown in Fig. 6 and Fig. 7. In contrast, comparatively lower VFA levels in the methanogenic reactor indicate efficient downstream utilization of these intermediates. The gradual decrease in VFA concentration in the methanogenic reactor suggests effective syntrophic interactions between fermentative bacteria and methanogens between fermentative bacteria and methane-producing archaea, thereby contributing to overall process stability.

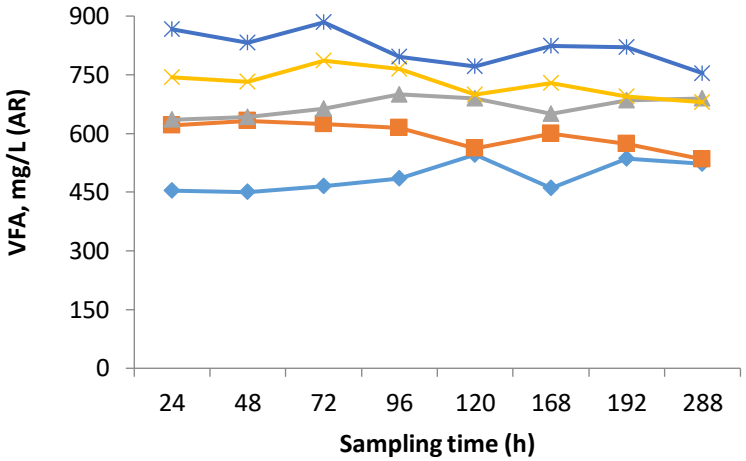


Fig. 6: Effect of sampling time (h) on VFA, mg/l in Acidogenic reactor (AR)

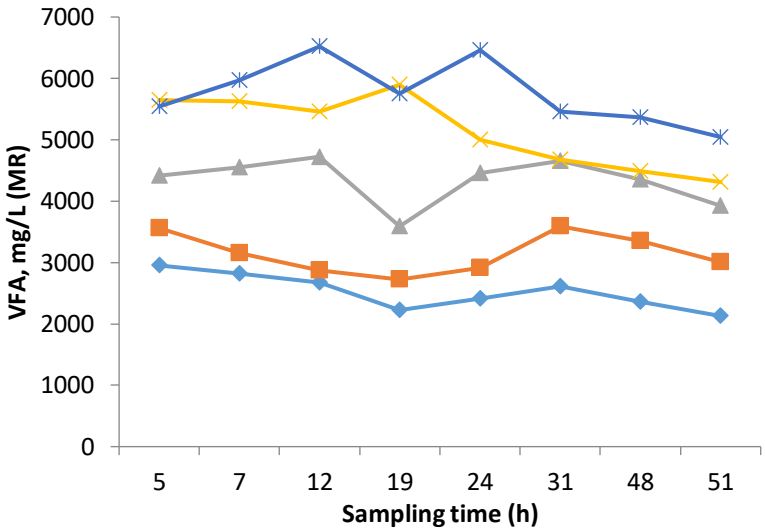


Fig. 7: Effect of sampling time (h) on VFA, mg/l in Methanogenic reactor (MR)

4.4.3. Biomass Retention (VSS Profile)

The VSS concentration in AR varied from 509 to 2450 mg/L, reflecting suspended microbial growth. In MR, VSS values ranged between 378 and 1971 mg/L. It can be observed from the Fig. 8 and Fig. 9 that the sustained biomass concentration in MR indicates enhanced microbial retention due to the attached-growth mechanism supported by coconut coir. The fibrous and porous structure of coconut coir provided a favorable surface for biofilm development, minimizing biomass washout and improving reactor resilience during increased organic loading. The higher VSS observed in MR indicates improved microbial retention facilitated by the coconut coir bio-carrier.

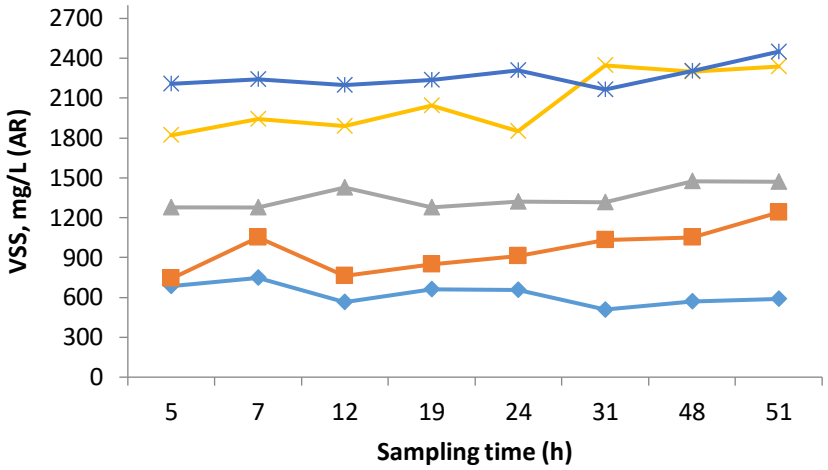


Fig. 8: Effect of sampling time (h) on VSS, mg/L in Acidogenic reactor (AR)

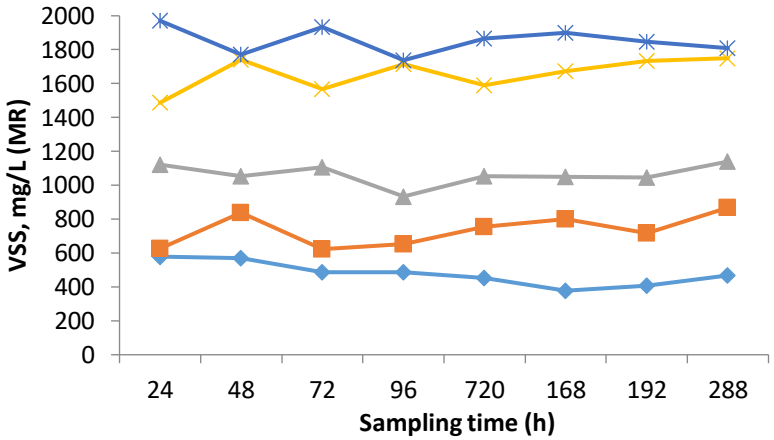


Fig. 9: Effect of sampling time (h) on VSS, mg/l in Methanogenic reactor (MR)

4.5. Influence of Loading Rate on COD Removal Efficiency

The effect of Organic Loading Rate (OLR) on reactor performance was assessed through COD removal efficiency in both phases. Applied influent OLR ranged from 0.5 to 3.558 kg COD/m³·d based on overall system loading. Due to smaller acidogenic reactor volume, phase-specific OLR in AR reached 21.14 kg COD/m³·d.

4.5.1. Acidogenic Reactor Performance

The maximum COD removal efficiency in AR was 27.39% at 1.809 kg COD/m³·d, while the minimum value of 15.78% was observed at 21.14 kg COD/m³·d. The relatively lower COD removal in AR as shown in Fig. 10 is

expected, as the primary function of this phase is substrate hydrolysis and acid production rather than complete mineralization.

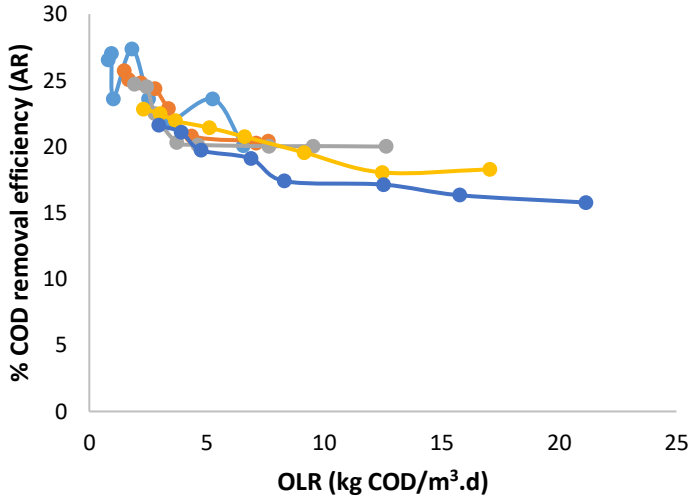


Fig. 10: Effect of OLR (kg COD/m^3.d) on % COD removal efficiency in Acidogenic reactor (AR)

4.5.2 Process Limitation at Higher Loading

Operation beyond the identified threshold OLR resulted in a measurable decline in COD removal efficiency, indicating temporary process stress. The accumulation of intermediate acids under higher loading likely created localized inhibitory conditions affecting methanogenic activity. However, the system maintained functional stability without complete process failure, suggesting that the performance reduction defines an operational boundary rather than structural instability. Establishing this loading threshold is essential for scale-up and industrial application of natural carrier-based biphasic anaerobic systems treating high-strength pharmaceutical effluents. Although high percentage cod removal was achieved, post-treatment polishing would be necessary to meet discharge standards.

4.5.3. Methanogenic Reactor Performance

The biphasic system showed distinct differences in biogas production between AR and MR. AR produced lower biogas yield (0.009–0.053 m^3 kg^{-1} COD removed), indicating its primary role in hydrolysis and acidogenesis, with moderate methane presence. MR exhibited higher biogas yield (0.047–0.090 m^3 kg^{-1} COD removed), confirming efficient methane production. Methane content increased and CO_2 decreased in MR, indicating enhanced methanogenesis under stable neutral pH. Overall, phase separation improved methane recovery and biogas quality. Biogas production and composition characteristics in Acidogenic and Methanogenic reactors under varying Organic Loading Rates are presented in Table 5.

Table 5: Biogas production and composition characteristics in AR and MR under varying Organic Loading Rates

Reactor	OLR (kg COD m^{-3} d^{-1})	Biogas Yield (m^3 kg^{-1} COD removed)	CH_4 (%)	CO_2 (%)
Acidogenic Reactor (AR)	0.811 – 6.549	0.009 – 0.016	55.14 – 59.70	29.34 – 30.72
	1.492 – 7.611	0.016 – 0.020	57.34 – 58.68	27.17 – 28.72
	1.933 – 12.64	0.023 – 0.030	59.72 – 60.85	25.24 – 27.07
	2.304 – 17.043	0.032 – 0.042	61.26 – 63.24	23.32 – 25.17
	2.947 – 21.140	0.043 – 0.053	63.39 – 65.72	22.19 – 23.23
Methanogenic Reactor (MR)	0.112 – 1.046	0.047 – 0.052	68.23 – 69.81	21.18 – 22.34
	0.221 – 1.210	0.052 – 0.055	69.90 – 71.46	19.27 – 21.09
	0.290 – 2.021	0.056 – 0.063	71.51 – 72.63	18.25 – 19.23
	0.355 – 2.042	0.064 – 0.074	72.71 – 73.70	17.15 – 18.12
	0.461 – 2.637	0.075 – 0.090	73.18 – 74.69	16.15 – 17.40

The MR exhibited significantly higher COD removal efficiency as depicted in Fig. 11, with a maximum of 87.54% at 0.462 kg COD/m^3·d. However, the efficiency decreased to 64.68% at 3.558 kg COD/m^3·d. The observed reduction in COD removal at higher loading rates suggests a temporary imbalance between acid formation and methane conversion, resulting in partial suppression of methanogenic activity. Similar reductions in methane production efficiency at elevated OLR have been reported in two-phase anaerobic systems (Demirel and Yenigun, 2002; Kumar et al. 2022). This suggests that the stable operating limit of the coconut coir–immobilized methanogenic reactor lies below approximately 2.5–3.0 kg COD/m^3·d.

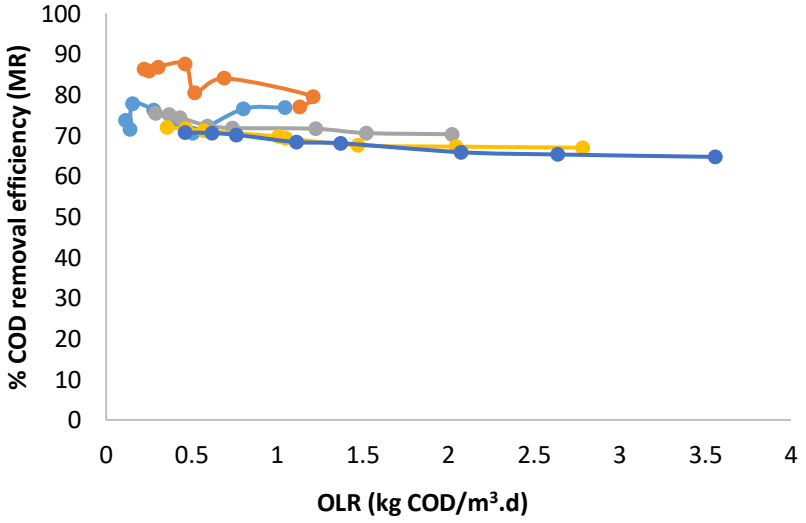


Fig. 11: Effect of OLR (kg COD/m^3.d) on % COD removal efficiency in Methanogenic reactor (MR)

5. CONCLUSIONS

The study demonstrated that a biphasic anaerobic reactor packed with coconut coir can effectively treat high-strength pharmaceutical wastewater under mesophilic conditions. Distinct functional separation between acidogenic and methanogenic stages was observed, supported by the presence of different microbial communities in each reactor phase.

The use of coconut coir as a natural support medium enhanced microbial attachment and reduced biomass washout, which contributed to stable reactor performance under increasing organic loading rates. Maximum COD removal efficiency of 87.54% was achieved at lower loading conditions, while moderate reductions were observed at higher loading rates due to temporary accumulation of volatile fatty acids.

Overall, the results suggest that natural lignocellulose materials such as coconut coir can be used as cost-effective carrier media for improving the performance and stability of anaerobic treatment systems treating industrial wastewater. Specific pharmaceutical compounds, antibiotics and toxicity indicators were not analyzed, therefore treatment performance reflects bulk organic matter removal only.

Author Contributions:

Kayalvizhi Natarajan: Conceptualization; Methodology; Experimental investigation; Data collection; Formal analysis; Microbial isolation and molecular analysis; Writing – original draft preparation; Visualization; Data interpretation.

Gurumoorthy Chandrasekharan: Supervision; Research design validation; Methodology refinement; Data validation; Critical review and editing; Interpretation of results; Project administration; Final manuscript approval.

All authors have read and approved the final manuscript.

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