Journal of Applied Research in Water and Wastewater

E-ISSN: 2476-6283

Journal homepage: https://arww.razi.ac.ir

Improvement of anaerobic sludge granulation and biogas production by bone powder as a natural material

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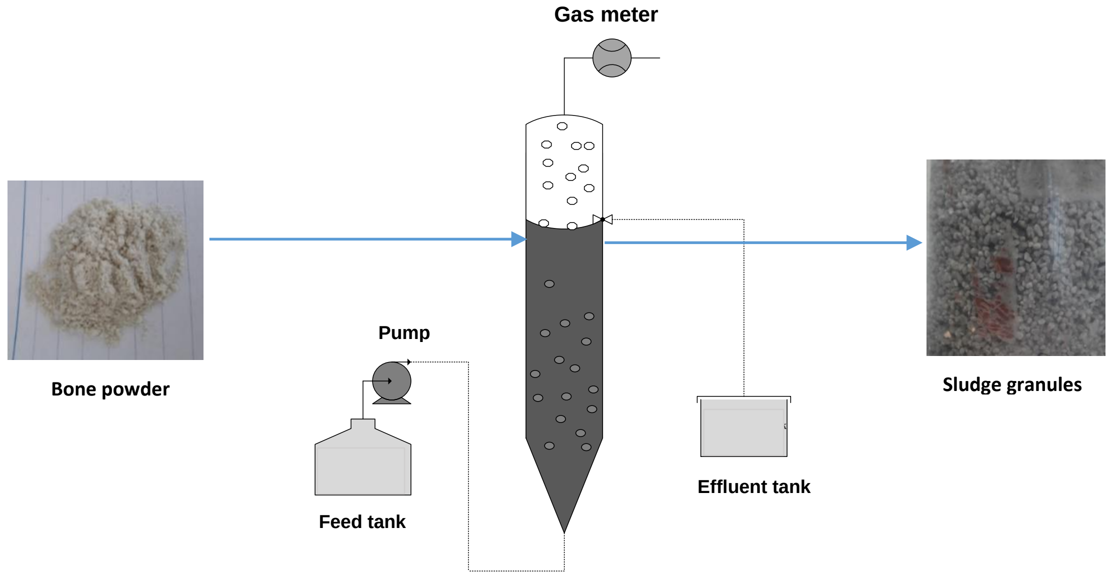
GRAPHICAL ABSTRACT



ARTICLE INFO

Article type:
Research Article

Article history:
Received 28 March 2024
Received in revised form 4 June 2024
Accepted 5 June 2024
Available online 6 June 2024

Keywords:
Up-flow anaerobic sludge blanket (UASB) reactors
Methane production
Bone powder
Biogas production

ABSTRACT

Soft drinks are industrial intermediate chemicals found in wastewater and are among the most significant environmental pollutants. Up-flow anaerobic sludge blanket (UASB) reactors are used to treat soft drink wastewater, offering high-volume loading capacity, optimal grain deposition, and the ability to bear impact loads. The use of support materials can enhance biological productivity and expedite the UASB start-up period. In this study, bone powder was utilized as a support material in a UASB reactor to remove contaminants from wastewater. During the 70-day sludge adaptation period, the treatment reactor achieved a 93% reduction in COD, while the control reactor achieved a 65% reduction. Biogas production was higher in the bone powder UASB reactor (1750 mL/d) compared to the control UASB reactor (1100 mL/d), and the bone powder UASB reactor demonstrated greater resistance to shock loading. The improvement in sludge settling, shear strength, and higher biological activity in the bone powder UASB reactor was attributed to the formation of large granular sludge. The size of the granular sludge increased further with the colonization of filamentous bacteria at the irregular levels of bone powder.

1. Introduction

In recent years, the growing trend of energy consumption has created a global energy crisis (Wang and Wan, 2009; Wang, Li, and Ren, 2013). Energy sector leaders must prioritize structural changes, switch energy carriers, and replace fossil fuels with cleaner alternatives (Arimi *et al.*, 2015). According to statistics released by the New Energy Organization in 2014, nearly 80 % of global energy demand is met by fossil fuels (oil, gas, and coal). The increasing consumption of these fuels results in the release of pollutants such as CO_x, NO_x, and SO_x, leading to greenhouse effects, stratospheric ozone depletion, acid rain, and environmental pollution. Studies worldwide have shown that clean energy production technologies have gained significant attention in many countries in recent years. Biogas, a renewable energy source, is produced from

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Cite this article: F. Falahi, P. Mohammadi, S.A. Mousavi, A. Arami-Niya, Improvement of anaerobic sludge granulation and biogas production by bone powder as a natural material, *Journal of Applied Research in Water and Wastewater*, 11 (1), 2024, 24-30. doi: https://doi.org/10.22126/arww.2024.10144.1319

organic waste in sanitary wastewater, industrial wastewater, and solid waste by anaerobic microorganisms. Anaerobic biological systems effectively purify and reduce the pollutant load of these wastes, resulting in the production of methane and hydrogen gases, which are sources of clean energy (Wu, Zhu, and Miller, 2013).

In anaerobic digestion, microorganisms convert organic matter into biogas through four stages: hydrolysis, acidification, fermentation, and methane production. Industries that require large amounts of water for production produce high volumes of polluted effluent, posing significant environmental risks, especially to groundwater resources. The beverage industry is one such example, consuming about 7 m^3 of water per cubic meter of beverage produced and generating wastewater with a high organic load (Yari *et al.,* 2005; Iraprasertwong, Maitriwong, and Chavadej, 2019). To address the increase in industrial effluent and the low treatment efficiency of traditional methods, the up-flow anaerobic sludge blanket (UASB) reactor was introduced in 1990. This method is highly suitable for treating heavily polluted effluents, as the high concentration of microorganisms allows for faster contaminant removal compared to other methods. In UASB systems, the efficiency of contaminant removal is directly related to the degree of sludge granulation within the reactor. However, the lack of protection for sludge inside the reactor can lead to the washout of low-density sludge, reducing the concentration of microorganisms and ultimately lowering treatment efficiency. Therefore, it is crucial to start the UASB reactor slowly to allow for sludge granulation, a process that typically takes 2 to 8 months and is considered the main weakness of UASB reactors (Siewhui, C. *et al.*, 2012).

Applying a nucleation component during the granulation process can significantly assist in reactor operation. Recent years have seen various modifications and considerations of different variables to enhance material removal efficiency and biogas production with new types of nucleation materials (Cooney *et al.,* 2007; Mohan, S.V. *et al.,* 2011). Researchers have found that materials such as hydroanthracite, organic polymers, sponges, and activated carbon significantly affect biomass granulation behavior (Lee, Lin and Chang, 2006; Wang, Z. *et al.*, 2006). Additionally, natural and synthetic cationic polymers such as chitosan and polyacrylamide (Ariyavongvivat, Suraraksa, and Chaiprasert, 2015), coagulants like aluminum chloride (Yu, Fang and Tay, 2001), multiple positive ions such as iron, calcium, magnesium, and aluminum (Zhang, An, and Quan, 2011), and different types of adsorbents like zeolite (Pérez-Pérez, *et al.,* 2017) have been used to improve granulation. Research has shown that cow bone powder is an effective adsorbent for removing heavy metals like cadmium from wastewater (Abdulrahman *et al.,* 2016). Another study demonstrated that fish bone powder could remove 198 reactive red dye with an efficiency of about 85% (Ghaneian, 2012). The mineral part of bone is composed of a bioactive and biocompatible ceramic compound called hydroxyapatite [19], which is widely used for wastewater treatment due to its ability to clean various hazardous heavy metals such as lead, cadmium, zinc, arsenic, vanadium, and uranium by replacing them with calcium ions in its crystalline structure [20].

However, no studies have investigated the effect of bone powder (natural hydroxyapatite) on granulation. This study aims to examine the impact of bone powder on nucleation and faster granule formation in bioreactors and methane production. This innovative study explores the use of different materials for granulation and methane production. Given the increasing population and industrial processes that lead to wastewater production and higher energy consumption, the system studied in this research is promising for addressing these challenges. The study aims to reduce the cost of wastewater treatment while maintaining the existing microbial population within the bioreactor, thus reducing the issue of mixed liquid excretion. Therefore, this study focuses on the biological production of methane in the UASB reactor from high organic load wastewater and investigates the efficiency of COD removal and the effect of using bone powder in granulation.

2. Materials and methods

Soft drink wastewater (SDW) was collected from a working soft drink production plant, Zamzam Co., Kermanshah, Iran. The wastewater had COD and pH characteristics of 180,000 mg/L and 6.8, respectively. The samples were stored at 4°C to preserve their characteristics.

To investigate the effect of bone powder (hydroxyapatite) on improving the granulation rate, four UASB reactors were constructed from plexiglass with a 6 cm diameter, a height of 50 cm, and a working volume of 1.4 L. The study was conducted as follows:
Reactor C (control): No bone powder added
Reactor No. 1: 200 mg/L of bone powder added
Reactor No. 2: 400 mg/L of bone powder added
Reactor No. 3: 600 mg/L of bone powder added

A grid was placed at the bottom of the reactors to distribute the current evenly. Wastewater was injected into the reactors using a peristaltic pump. Schematic images of the reactors are shown in Fig. 1. Anaerobic digestion sludge from the Sanandaj refinery was used to initially inoculate the reactors. After washing and smoothing the sludge to remove impurities, the VSS and TSS were measured, with the VSS/TSS ratio being approximately 0.5. The systems were set up with MLSS at 6000 mg/L, soft drink wastewater with COD at 1000 mg/L, a hydraulic retention time of 24 h, and an up-flow velocity of 1.2 m/h. The reactor temperature was maintained in the mesophilic range (35-37°C) using hot water flow in the reactor walls. Alkalinity was adjusted to 2000 mg/L by adding sodium bicarbonate. Nutrients were added in a COD:N ratio of 200-350:5:1. The pH was adjusted in the range of 7.5-7.2. The up-flow velocity, initially set at 1.2 m/h, gradually increased to 3 m/h. After a 3-week adaptation period for the microorganisms to the wastewater, bone powder (Fig. 1) was added to the system with a mesh size of 50-100. The COD concentration gradually increased from 1000 to 8000 mg/L. COD, pH, temperature, daily and total gas volume, and the percentage of gas compounds were controlled and measured twice a week. Gas volume was measured using a gas meter, and the percentage of gas compounds was measured using a GC device. Granules were sampled once a week to check their size. Standard methods were used for determining COD and MLSS (APHA, 1926). For COD, a colorimetric method with a closed reflux method was developed (D1252). A spectrophotometer (DR 5000, Hach, Jenway, USA) was used at 600 nm for COD concentration measurements.

3. Results and discussion

3.1. Examination of granules in the system

To evaluate the effect of bone powder, the size and formation time of granules in the four reactors were investigated. Granules are defined as particles with a diameter greater than 0.125 mm. Granules with a diameter of 0.2-0.3 mm were observed one week after the addition of bone powder in reactors R1, R2, and R3, and two weeks later in reactor C.

Over time, the distribution and diameter of the granules increased. Fig. 2 illustrates the condition of the reactor granules before and after the addition of bone powder. As shown in Fig. 2, granules in reactor C were smaller and less distributed compared to the other reactors. One month after the addition of bone powder, the diameter of granules in reactor C was about 1 mm or less, whereas in the other reactors, granule diameters ranged from 0.5 to 3 mm. Among all the reactors, reactor R2 (containing 400 mg/L bone powder) exhibited the highest granule distribution and size. These granules demonstrated better sedimentation and removal efficiency. Bone powder appears to act as a nucleus for microorganisms to coalesce, thus improving granule formation. The release of calcium from bone powder may also contribute to the rapid formation of granules. Fig. 3 shows an image of reactor R2 after two months, with granules ranging from 1 to 4 mm in diameter. However, at the end of the lead time, the granule size in all reactors decreased slightly due to the high organic load and increased gas production. This observation aligns with the results of a study by Hudayah et al. (2012), in which polyacrylamide was used to enhance granulation in a UASB reactor. Their results indicated that after 69 days, the average granule size was 126 μm, compared to 72 μm in the control reactor, indicating that polyacrylamide increased the granule size by approximately 1.75 times. They also observed a decrease in granule size with an increasing organic load (Hudayah, Suraraksa, and Chaiprasert, 2012).

Comparing these results, it is evident that in the present study, after one month, the average granule size in the treated reactors was more than twice that of the control reactor, demonstrating the efficiency of bone powder in enhancing and accelerating granulation. In a 2001 study by Yu et al., the addition of aluminum chloride to a UASB reactor resulted in granulation after 35 days, with granule sizes ranging from 0 to 4.2 mm after 60 days (Yu, Fang, and Tay, 2001). In another study, Chen et al. investigated the effect of turf soil on the granulation rate in a UASB reactor, finding that the average granule size in the treated reactor was 0.06-0.06 mm, compared to the control reactor (Chen, 2018). Ariyavongvivat et al. (2015) reported that the application of chitosan in a UASB reactor improved the physical, chemical, and biological properties of the granules (Ariyavongvivat, Suraraksa, and Chaiprasert, 2015). Sanjeevi et al. (2012) studied the effect of calcium ions on granulation in a UASB reactor, concluding that the granulation process occurred faster, and larger granules formed compared to the control reactor.

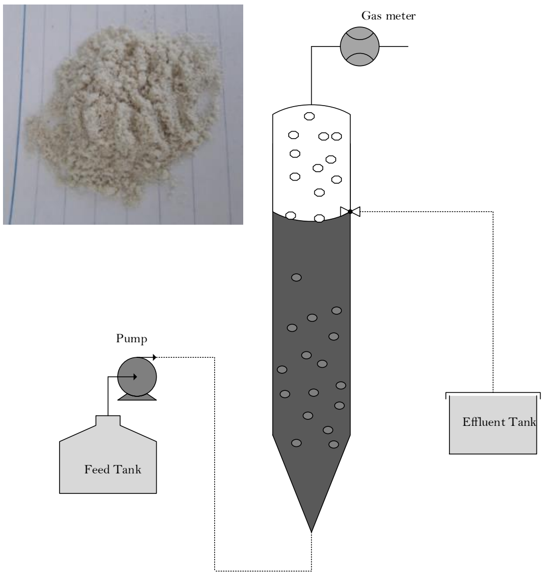


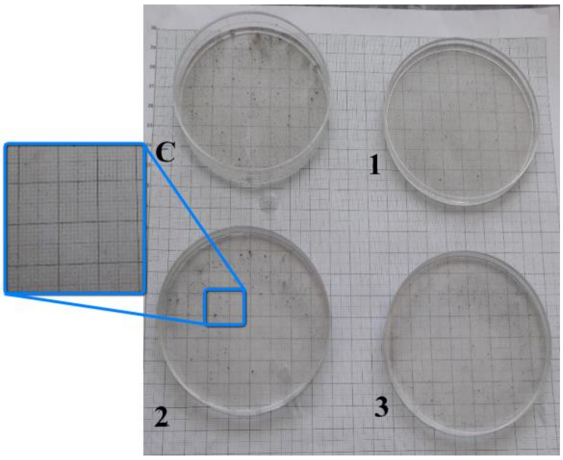
Fig. 1. Schematic of the reactor and real bovine bone mineral powder.

3.2. Evaluation of COD removal efficiency

COD removal was measured as a parameter to evaluate the performance of the reactors. The initial input COD in the sludge acclimation stage with wastewater was 1000 mg/L, which gradually increased over 70 days to 8000 mg/L in all tests. The hydraulic retention time was 24 h, and the up-flow velocity was initially 1.2 m/h, gradually increasing to 3 m/h. The results of the experiments and the COD removal efficiency of the reactors in the range of 1000 to 8000 mg/L are shown in Figs. 4 and 5.

During the acclimation stage, the COD removal efficiency of all reactors was almost the same, with an average removal rate of more than 60%, as shown in Fig. 5b. After the acclimation stage (21 days), the input COD was increased by adding bone powder. As shown in Figs. 4 and 5, reactor C (without bone powder), which had smaller and less distributed granules, exhibited lower COD removal efficiency compared to the other reactors. In reactor C, due to the late formation of granules, the removal efficiency reached an acceptable level later. After 40 days, the COD removal efficiency of reactor C reached 80%, while in the other reactors, the removal efficiency surpassed 80% after just 27 days.

Comparison of the results shows that reactors R1, R2, and R3 achieved better and faster purification than reactor C. Sampling indicated that in these reactors, granule formation was faster, larger, and less dispersed than in reactor C. Among reactors R1, R2, and R3, reactor R2, with 400 mg/L of bone powder, consistently showed the highest COD removal efficiency under all conditions. The highest COD removal rate was about 96.1% in reactor R2 with an input COD concentration of 5000 mg/L after 45 days, while under the same conditions, reactor C's removal efficiency was about 82%. When the input COD increased to 6000 to 8000 mg/L, the removal efficiency of all reactors decreased, possibly due to the rapid increase in input COD [23]. Compared to the other reactors, reactor C's efficiency decreased more sharply. At the end of 70 days, with an input COD of 8000 mg/L, reactor C's removal efficiency dropped to 50%, whereas the optimal reactor (R2) maintained a removal efficiency of about 78%. This indicates the effect of bone powder on the resistance of granules to organic load shocks.



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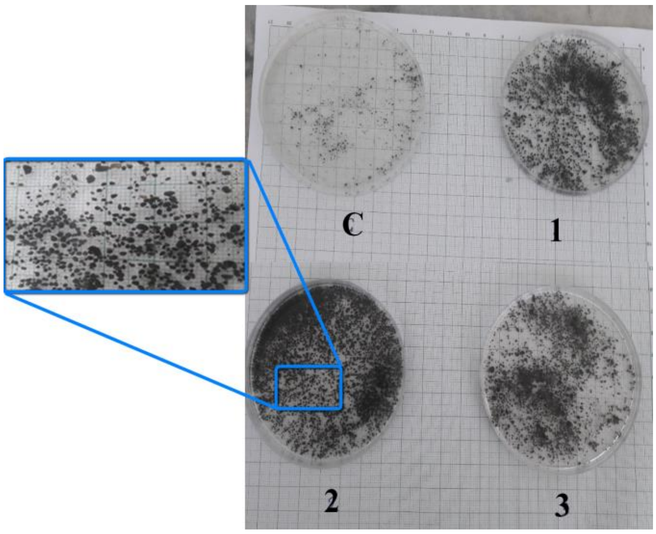


(b)

Fig. 2. Status of reactor granules (a) before the increase of bone powder (b) one month after the increase of bone powder.

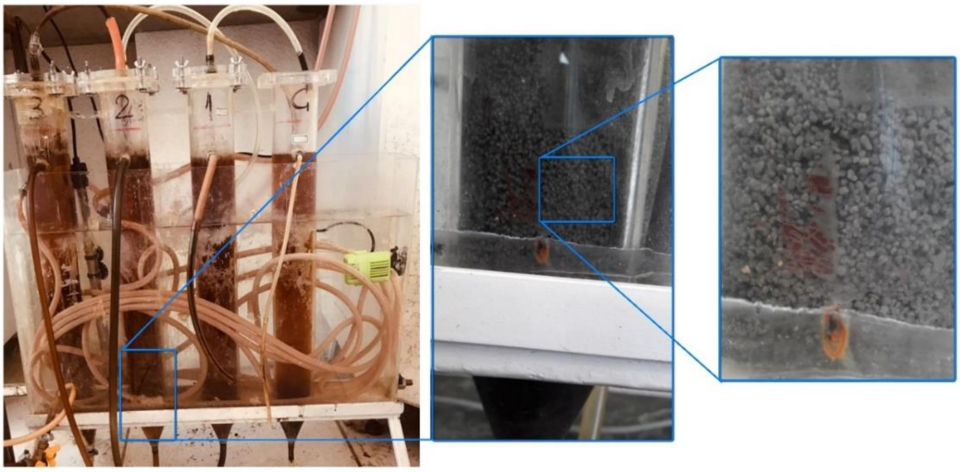


Fig. 3. Images of R2 reactor granules 40 days after the increase in bone powder.

The results of this study are consistent with those of Yeow et al. (2004), who found that improved granulation due to the addition of polymer led to improved filtration and increased removal efficiency in the UASB reactor (Show *et al.*, 2004). A study by Yu et al. (2001) also showed that the addition of aluminum chloride to the UASB reactor improved granulation, resulting in a faster granulation process and a removal rate of almost 90% [13]. Similarly, Chen et al. (2018) found that using turf soil in the UASB reactor to improve granulation resulted in higher COD removal efficiency and greater shock resistance compared to the control reactor (Chen, 2018).

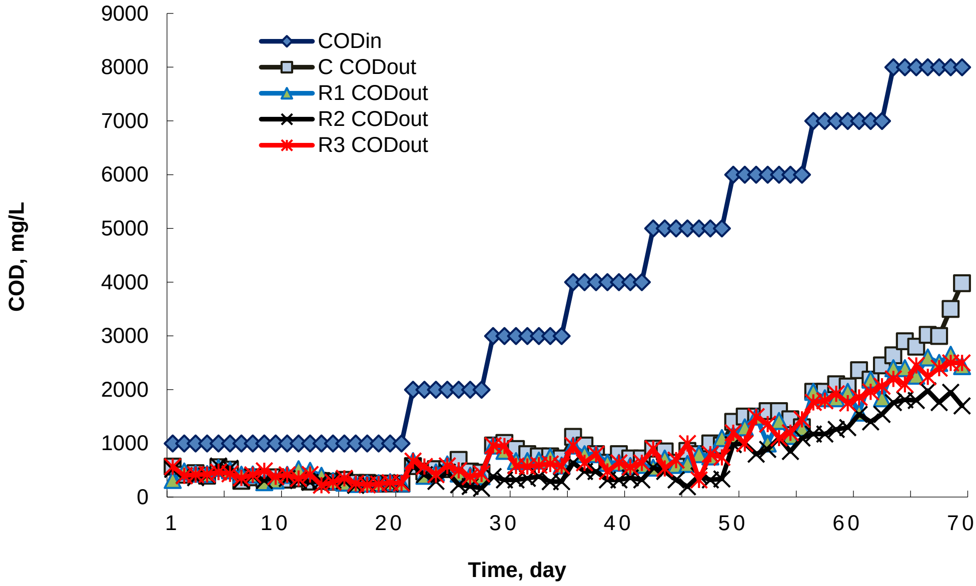
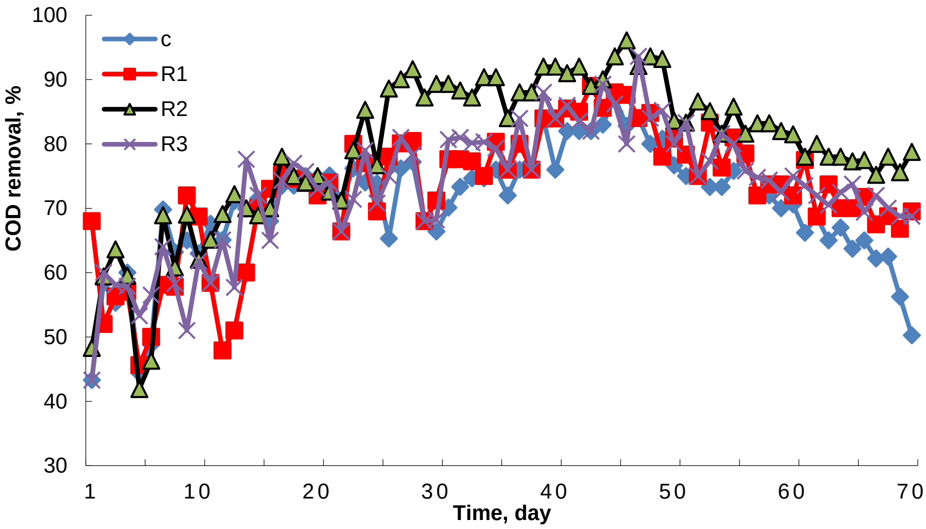


Fig. 4. The trend of increasing the input COD rate and COD output of reactors.



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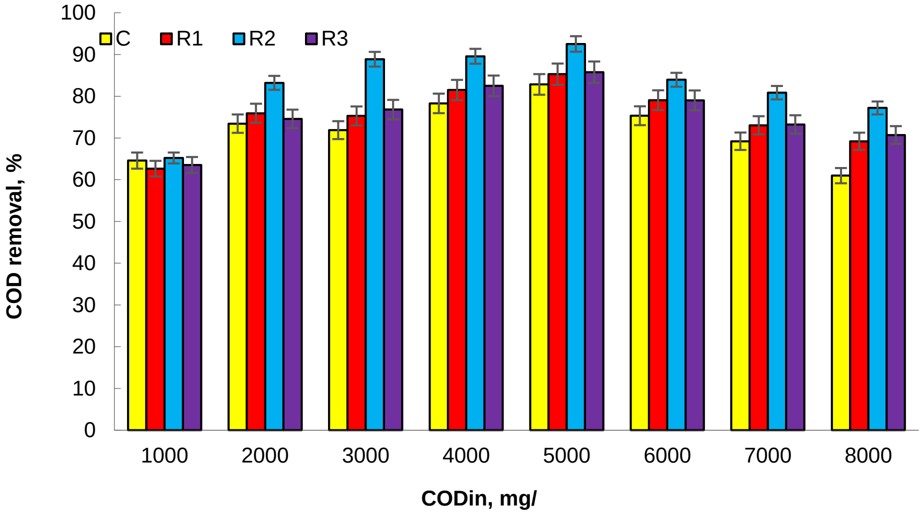


(b)

Fig. 5. (a) Average COD removal at different concentrations of reactor input COD and (b) COD removal efficiency (COD_{in} = 1000-8000 mg/L).

3.3. Check gas production

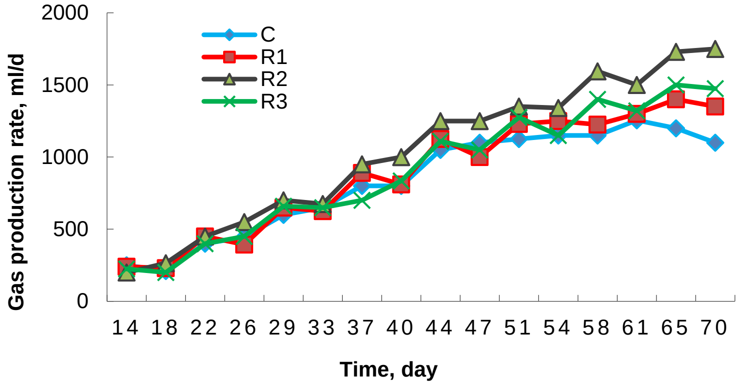
The biogas production and methane percentage in the reactors were monitored twice a week over two weeks after the reactors were initiated. Biogas production consisted of methane, carbon dioxide, and nitrogen, with 350 mL of methane theoretically produced per gram of COD removed. Fig. 6a and b illustrate the biogas production during reactor operation at varying input COD concentrations. It is evident that as the input COD concentration increased over time, biogas production gradually increased.

Initially, the biogas production rate was similar across all reactors, but as the input COD concentration increased, reactors R1, R2, and R3 consistently showed higher biogas production rates compared to reactor C. The biogas production rate after 70 days ranked as R2 > R3 > R1 > C. Specifically, biogas production rates from day 14 to 70 were approximately 250-1100 mL/day for C, 240-1350 mL/day for R1, 200-1750 mL/day for R2, and 225-1475 mL/day for R3.

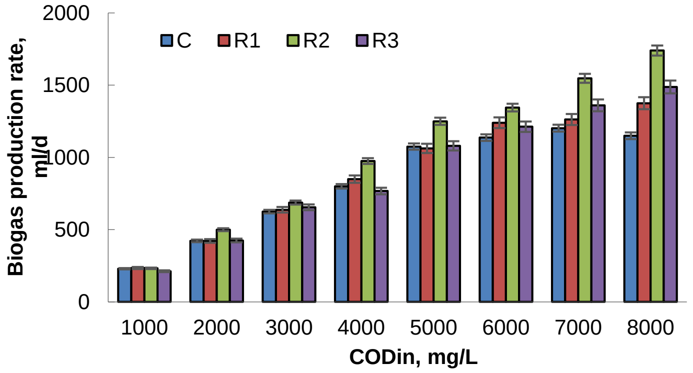
Comparing these results indicates that the addition of bone powder improved biogas production due to the formation of stable and larger granules. Notably, reactor R2 exhibited the highest biogas production, reaching about 37% higher than reactor C at an input COD concentration of 8000 mg/L over 70 days. This increase can be attributed to the COD consumption by reactor R2 and the distribution of its granules.

Fig. 6c shows the methane production percentage during the operation of the reactors. Over time, the percentage of methane production increased and stabilized. Approximately 14 days after initiating the reactors, methane production exceeded 65% and gradually rose above 80%. Reactor C showed a slower increase in methane production percentage compared to the other reactors, reaching 80% about 60 days after commissioning. The highest methane production percentage, around 85%, was observed in reactor R2 after approximately 40 days.

These findings suggest that granule formation significantly influences methane production efficiency, with reactors exhibiting larger and better-formed granules producing more methane. This observation aligns with the study by Chen et al. (2018), which demonstrated that enhancing granule formation in UASB reactors, such as by adding turf soil, increases microbial activity and consequently biogas production (Chen, 2018). Similarly, Zhang et al. (2011) reported positive effects of iron additives on UASB reactor granulation and methane production (Zhang, An, and Quan, 2011).



(a)



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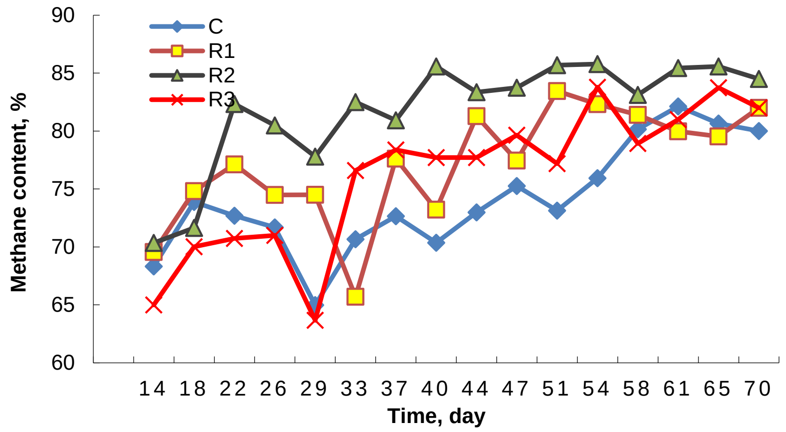

(c)

Fig. 6. (a) Production gas during the operation time of reactors, (b) mean of biogas produced at different concentrations of input COD and (c) percentage of methane production during the operation of reactors.

4. Conclusions

This study highlights bone powder as a natural source of hydroxyapatite that can effectively enhance the performance of UASB reactors in treating industrial wastewater. By acting as a nucleation agent, bone powder facilitated the rapid formation of large and robust granules in less than a month. These granules not only improved removal efficiency of pollutants but also significantly increased biogas production rates and methane content. The substantial production of biogas, particularly with a high methane percentage, offers a viable avenue for energy generation. The optimal concentration of bone powder identified in this study was 400 mg/L. Bone powder is advantageous due to its natural origin, widespread availability, and cost-effectiveness, making it a promising material for enhancing the efficiency of wastewater treatment in UASB reactors.

Author Contributions

Farzaneh Falahi: Conceptualization, investigation, methodology.
Parviz Mohammadi: Writing original draft, supervision, validation.
Seyyed Alireza Mousavi: Investigation, methodology, analysis, review, and editing.
Arash Arami-Niya: Review and editing.

Conflict of Interest

I declare that all authors have read and approved submission of the manuscript, and that material in the manuscript has not been published and is not being considered for publication elsewhere in whole or in part in any language. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to acknowledge Kermanshah University of Medical Sciences, Kermanshah, Iran (project no. 95489) for the full financial support provided for this research work.

Data Availability Statement

Data will be made available on request.

References

Abdulrahman, A. *et al.* (2016) 'Preparation and characterization of activated cow bone powder for the adsorption of cadmium from palm oil mill effluent. In IOP Conference Series: Materials Science and Engineering', 136 (1), p. 012045. IOP Publishing. doi: https://doi.org/10.1088/1757-899X/136/1/012045

Siewhui, C. et al. (2012) 'The performance enhancements of upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment – A State-of-the-art review'. Water Research, 46, pp 3434-3470. doi: https://doi.org/10.1016/j.watres.2012.03.066

,*American Public Health Association (APHA), 1926. Standard methods for the examination of water and wastewater (Vol. 6). American Public Health Association.*

Arimi, M.M. *et al.* (2015) 'Strategies for improvement of biohydrogen production from organic-rich wastewater: a review', *Biomass and bioenergy*, 75, pp.101-118. doi: https://doi.org/10.1016/j.biombioe.2015.02.011

Ariyavongvivat, E., Suraraksa, B. and Chaiprasert, P. (2015) 'Physicochemical and biological characteristics of enhanced anaerobic microbial granulation by synthetic and natural cationic polymers', *Energy Procedia*, *79*, pp. 851-858. doi: https://doi.org/10.1016/j.egypro.2015.11.577

Chen, C. (2018) 'Turf soil enhances treatment efficiency and performance of phenolic wastewater in an up-flow anaerobic sludge blanket reactor', *Chemosphere*, 204, pp. 227-234. doi: https://doi.org/10.1016/j.chemosphere.2018.04.040

Cooney, M. *et al.* (2007) 'Two-phase anaerobic digestion for production of hydrogen–methane mixtures', *Bioresource Technology*, 98 (14), pp. 2641-2651. doi: https://doi.org/10.1016/j.biortech.2006.09.054

Ghaneian, M.T. (2012) 'A survey of the efficacy of cuttle fish bone powder in the removal of Reactive Red 198 dye from aqueous solution", *Tolooebehdasht*, 10 (3-4), pp. 127-138. Available at: http://tbj.ssu.ac.ir/article-1-1805-en.html (Accessed date: 20 July 2024).

Hudayah, N., Suraraksa, B., and Chaiprasert, P. (2012) 'The effect of synthetic cationic polymer addition on nucleation of anaerobic granules under different organic loading rate', *International Journal of Bioscience, Biochemistry and Bioinformatics*, 2(4), p.264-269. doi: https://doi.org/10.7763/IJBBB.2012.V2.113

Jiraprasertwong, A., Maitriwong, K., and Chavadej, S. (2019) "Production of biogas from cassava wastewater using a three-stage upflow anaerobic sludge blanket (UASB) reactor', *Renewable energy*, 130, pp. 191-205. doi: https://doi.org/10.1016/j.renene.2018.06.034

Lee, K.S., Lin, P.J. and Chang, J.S. (2006) 'Temperature effects on biohydrogen production in a granular sludge bed induced by activated carbon carriers', *International Journal of Hydrogen Energy*, *31*(4), pp. 465-472. doi: https://doi.org/10.1016/j.ijhydene.2005.04.024

Mohan, S.V. *et al.* (2011) 'Firmicutes with iron dependent hydrogenase drive hydrogen production in anaerobic bioreactor using distillery wastewater', *international Journal of Hydrogen Energy*, 36 (14), pp. 8234-8242. doi: https://doi.org/10.1016/j.ijhydene.2011.04.021

Pérez-Pérez, T. *et al.* (2017) 'Effects of the support material addition on the hydrodynamic behavior of an anaerobic expanded granular sludge bed reactor', *Journal of Environmental Sciences*, *54*, pp.224-230. doi: https://doi.org/10.1016/j.jes.2016.02.011

Show, K.-Y., *et al.*, (2004) 'Accelerated start-up and enhanced granulation in upflow anaerobic sludge blanket reactors', *Water*

Research, 38 (9), pp. 2293-2304. doi: https://doi.org/10.1016/j.watres.2004.01.039

Wang, B., Li, Y., and Ren, N. (2013) 'Biohydrogen from molasses with ethanol-type fermentation: effect of hydraulic retention time' *International Journal of Hydrogen Energy*, 38 (11), pp. 4361-4367. doi: https://doi.org/10.1016/j.ijhydene.2013.01.120

Wang, J., and Wan, W. (2009) 'Experimental design methods for fermentative hydrogen production: A review', *International journal of hydrogen energy*, *34*(1), pp.235-244. doi: https://doi.org/10.1016/j.ijhydene.2008.10.008

Wang, Z. *et al.* (2006) 'Effects of extracellular polymeric substances on aerobic granulation in sequencing batch reactors', *Chemosphere*, *63* (10), pp.1728-1735. doi: https://doi.org/10.1016/j.chemosphere.2005.09.018

Wu, X., Zhu, J., and Miller, C. (2013) 'Kinetics study of fermentative hydrogen production from liquid swine manure supplemented with glucose under controlled pH', *Journal of Environmental Science and Health, Part B*, *48*(6), pp.477-485. doi: https://doi.org/10.1080/03601234.2013.761907

Yari, A.R. *et al.* (2005) 'The efficiency of upflow anaerobic sludge blanket (UASB) in soft drink industry wastewater treatment', *Journal of Water and Wastewater*, *55*, pp. 31-38. doi: https://doi.org/10.1016/j.renene.2018.06.034

Yu, H.Q., Fang, H.H.P. and Tay, J.H. (2001) 'Enhanced sludge granulation in upflow anaerobic sludge blanket (UASB) reactors by aluminum chloride.', *Chemosphere*, 44 (1), pp.31-36. doi: https://doi.org/10.1016/S0045-6535(00)00381-7

Zhang, Y., An, X., and Quan, X., (2011) 'Enhancement of sludge granulation in a zero valence iron packed anaerobic reactor with a hydraulic circulation', *Process Biochemistry*, 46(2), pp.471-476. doi: https://doi.org/10.1016/j.procbio.2010.09.021