SCIENTIFIC REPORTS natureresearch

OPEN

Improvement of Co-Composting by a combined pretreatment Ozonation/Ultrasonic process in stabilization of raw activated sludge

Hamideh Dastpak^{1,2}, Hasan Pasalari^{1,2}, Ahmad Jonidi Jafari^{1,2}, Mitra Gholami^{1,2} & Mahdi Farzadkia^{1,2*}

The enhancement of composting technology to stabilize sludge pretreated by ozonation and ultrasonic was tested for 35 days. Secondary sludge produced by biological process are characterized with endogenous residue and inert solid matter which inhibit fully degrade bacterial cell walls. The composting process was performed with sludge pretreated with ozonatian and ultrasonics and green waste in a ratio of 2:1. The composting characteristics was evaluated for different physico-chemical and microbiological parameters in five different reactors. A high degree of composting quality was achieved with respect to significant reduction in volatile solids (VS) (32%), total organic carbon (TOC) (35.0%), C/N ratio (23.74), total coliform (TC) (168) along with the substantial increase in availability of nutrients like N (1.2%) and P (8.77%). High removal efficiency of TC and Fecal Coliform (FC) were observed in composting results, where simultaneous ultrasonic and ozonation were considered as primary-stabilization process. Therefore, applying integrated ultrasonic/ozonation with composting system for sludge stabilization is potentially useful technology in sustainable land restoration practices to meet standards and produce soil conditioner.

Overproduction of sewage sludge (SS), as an inevitable byproduct from activated sludge process, has currently led to use of inappropriate disposal practices¹. In most developing countries, the traditional disposal methods including landfilling and open-dumping are being applied². However, the presence of organic matter and highly polluted contaminants such as hazardous materials (i.e. antibiotics, bacteriostat, and flocculants) and heavy metals along with arbitrary discharge into environment have caused great concern about sludge management^{3,4}. Challenges associated with sewage sludge require to be addressed. Improper SS disposal methods could cause secondary pollution containing pathogenic microbes, heavy metals and organic micro-pollutants which induce plant toxicity and alter the metabolism of microorganism present in soil culture^{5,6}. Therefore, SS needs to undergo additional stabilization to decompose organic matter and reduce heavy metals to a recommended extent. On industrial scale, landfilling and incineration are recognized to be two environmentally accepted means of sewage sludge disposal⁷. However, the main drawback of these methods is that landfilling and incineration eliminate and remove the organic matter which may be considered for good uses^{2,8}. Hence, there is utmost need to select methodology ecologically sound, economically viable and socially acceptable9. Composting and vermicomposting are well-known as ecologically and economically sustainable technologies for converting biosolids into safe and stable compost, which can be applied for subsequent uses like agriculture^{10,11}. Microorganisms present in composting process accelerate organic degradation under controlled conditions; thermophilic stage enables sanitization of wastes and elimination of toxic compounds9. During composting, objectionable odors disappear quickly and the population of pathogenic microorganism reduce considerably⁸. Resultant products of composting are used as fertilizer in land application¹⁰. However, biooxidation processes may be inhibited by difficulty in degradation of the

¹Research Center for Environmental Health Technology, Iran University of Medical Sciences, Tehran, Iran. ²Department of Environmental Health Engineering, School of Public Health, Iran University of Medical Sciences, Tehran, IR, Iran. *email: farzadkia.m@iums.ac.ir

Physico-chemical and Microbial characteristics	Green waste	Raw sludge	Ozonized Sludge	Ultrasonic Sludge	Combined Ozonized/ Ultrasonic sludge
pH	7.30 ± 0.05	7.59 ± 0.04	7.2 ± 0.1	7.5 ± 0.08	7.35 ± 0.03
TS ^a	30.59 ± 0.10	2.59 ± 0.18	18.10 ± 1.2	17.92 ± 0.92	17.05 ± 0.35
VS/TS ^a	79.11 ± 0.08	89.18 ± 0.15	67 ± 0.13	67.18 ± 0.05	77.97 ± 0.07
TOC ^a	33.95 ± 0.12	39.55 ± 0.23	37.32 ± 0.15	37.35 ± 0.17	43.31 ± 0.14
TN ^a	0.78 ± 0.02	1.5 ± 0.03	1.05 ± 0.03	1.8 ± 0.08	1.1 ± 0.10
C:N Ratio	43.52 ± 0.37	26.37 ± 0.06	35.44 ± 0.14	20.73 ± 0.05	39.37 ± 0.01
TP ^a	8.5 ± 0.06	9.5 ± 0.10	2.8 ± 0.02	2.9 ± 0.03	2.5 ± 0.01
MC ^a	69.41 ± 0.69	97.50 ± 050	81.90 ± 0.75	82.08 ± 0.78	83.03 ± 0.12
TC ^b	400	1100	450	920	860
FC ^b	50	805	150	750	520

Table 1. Initial physico-chemical and microbial properties of green waste, raw sludge provided from MWTT,ozonized, ultrasonic and combined ozonation/ultrasonics sludge. ^aWeight in %. ^bMPN/g ds.

.....

structural compounds⁷. A variety of chemical and physical treatment techniques have been applied to enhance lysis and disintegration of sewage sludge, including thermal hydrolysis¹², mechanical disintegration¹³, freezing¹⁴, ultrasonic process^{15,16} and ozonation^{17,18}. One of the preferred technologies for sludge degradation is ozonation. Ozonation decomposes the contaminants and high molecular weight compounds present in the sludge to low molecular structure. In addition, ozone can be converted to oxidizing radicals (i.e., hydroxyl radicals) and react with organic and inorganic fractions of sludge^{19,20}. It can also oxidize the refractory compounds and convert them to biodegradable low-molecular compounds²⁰. Ultrasonic is currently recognized as highly effective, environmentally friendly and cost-effective pretreatment method in sewage sludge management^{16,21}. This process produces extreme condition, so-called, "hot-spots" with high temperature and pressure (5000 K, 1000 atm)²². Ultrasonic can disrupt the sludge flocks and allow the intracellular organic matter to extract from microorganism. Ultrasonic attack to unbounded and bonded water and increase total solid required for composting process with least energy expenditure²³. Ultrasonic brings about sludge reduction following solubolization organic matter and enhance sludge biodegradability. Recently, many researchers have incorporated ozonation for sludge disintegration and improve COD Solubilization^{19,24-27} found that ozonation could considerably disintegrate sludge and ozonated sludge had high solubilized COD, which improve the sludge digestion. Zhang et al.²⁸ observed that polysaccharides and proteins concentrations in supernatants were increased considerably after ozonation from 4.46 ± 0.21 mg/L to 220.90 ± 24.87 mg/L and 6.26 ± 0.28 mg/L to 386.54 ± 32.15 mg/L, respectively. Cheng *et al.*²⁹ reported that ultrasonic, as pretreatment for sludge stabilization decrease oxidation-reduction potential in aerobic reactors and improve the release of intracellular substances into environment, which consequently enhance biodegradability. Kavitha et al²³ reported a 60% dryness and dewatering can be obtained using ultrasonic without heat treatment. In addition, limited studies have focused on sludge stabilization using a combined ultrasonic and ozonation (Pei et al.)^{20,30,31} reported 30% and 28.10% sludge solubilization after applying ultrasonics (150,000 kJ/ kg TS) and ozonation (0.1 g O_3 /g TS), respectively. To the best of our knowledge, there are paucity of data concerning application of simultaneous ultrasonic in low frequency and ozonation process for biostabilization SS with composting process. Furthermore, most studies mentioned above utilized these processes as final sludge treatments. Therefore, the present research was developed for the first time to examine the performance of ozonation and ultrasonics as pretreatment in variations of physico-chemical and microbiological parameters of sludge. Additional aim of present research was to examine the ability of co-composting process in stabilization of raw activated sludge pretreated by ultrasonic and ozonation separately and simultaneously were evaluated.

Results and Discussion

Physico-chemical and microbiological parameters of sludge. The effects of ozonation on physio-chemical and microbiological characteristics of sludge were examined in consumed dose concentration of 6 g/L. As presented in Table 1, most physico-chemical properties of ozonated sludge including pH (7.2), TOC (37.32%), VS (67%), TN (1.01%), and TP (2.80%) had less values compared to initial ones. The findings of this primary-stabilized method are confirmed by other studies^{28,32}. After ozonation and disintegration of sludge, C, N, and P were placed in micro-solid phase which are good source for microorganisms functions. Many studies suggest that ozone attack microbial cells and finally release the intracellular content^{18,33}. Total (450) and Fecal colliform (150) of raw sludge decreased considerably after applying ozone. The operating conditions for ultrasonic were selected based on pretests and literature review. As shown in Table 1, a considerable reduction was observed in pH (7.5), VS (67.18%), TOC (37.35%), and on the other hand TN (1.8%) was increased after that sludge was exposed to ultrasonic radiation and all findings are confirmed by^{23,29}. The results indicated that ultrasonic pretreatment favors sludge disintegration and improve the biodegradability process. The ultrasonic process didn't change considerably TC (920) and FC (750), as microbiological parameters associated with sludge. It is noteworthy that the causes of variation of other parameters need further research and considerations. The sludge characteristics after applying simultaneous ozone and ultrasonic for primary-stabilization of raw sludge show that these parameters are in average values between values of ozonation and ultrasonic alone, except VS (77.97%), TOC (43.31%), and C/N (39.37%) ratio which had higher values compared to separate application of ozone and ultrasonic.



Figure 1. Variation of pH (A), TOC (B), VS (C), TN (D), C/N (E), TP (F) during composting stage of waste activated sludge.

Physico-chemical and microbiological analysis of composting stage. *pH.* An increase in pH of composting process under all experimental conditions were observed, as presented in Fig. 1(A). The maximum and minimum increased trends of pH were in reactors T2 (Initial: 7.59, Final: 8.59) and T4 (Initial: 7.20, Final: 7.98), respectively. The increasing trend during composting process corroborates with the results reported by other relevant studies^{1,9,34}. An increase in the pH of final composting process are assumed to be due to excess amount of mineral nitrogen content of substrate, changes in ammonium-nitrate equilibrium³⁵. According to Kruskal-Wallis statistical test and probability 95%, no significant difference was observed between mean pH of all different reactors studied in this work (p-value > 0.05).

Total organic carbon (TOC) and volatile solids (VS). A significant loss of total organic carbon (TOC) and volatile solids (VS) are important indications for decomposition, mineralization and maturity of composting process. In

Microbiological parameters	T1	T 2	T 3	T 4	T 5
тс	240	430	75	350	168
FC	35	250	36	185	23

 Table 2. Microbiological parameters of resultant composting process.

five different reactors, there was a significant reduction of TOC (35.1%), as presented in Fig. 1(B). The maximum and minimum percentage of reduction in TOC among reactors belonged to T5 (Initial: 40.7%, Final: 26.45%) and T1 (Initial: 33.95, Final: 29.19), respectively. The decrease of TOC percentage in the present study are in line with findings reported by other researchers^{2,36}. The reduction of TOC and VS are assumed to be results from loss of CO_2 following microbial respiration during composting process. Based on results from Kruskal-Wallis statistical test, there were significant differences among different reactors (p-value < 0.05). As for VS in reactors, there was a significant reduction of VS (32.0%), as presented in Fig. 1(C). The maximum and minimum percentage of reduction in VS among reactors belonged to T5 (32%) (Initial: 71.1%, Final: 48.2%) and T1 (14%)(Initial: 79.11%, Final: 68.03%), respectively. These significant reductions in the VS values are one of considerable indications for sludge stabilization. The findings obtained associated with VS reduction in the present study were higher than other research reported by⁹ (29.1%) which examined VS after composting process.

Total nitrogen (TN). A considerable increase in TN content was observed in all reactors. The increase in TN content during composting process was in range 0.03–100%. However, the maximum TN value was achieved in T4 with 1.52%. During sludge composting process, some biochemical reactions affect the TN in the process such ammonification, ammonia assimilation, nitrification and denitrification as fluctuate TN values³⁷. As presented in Fig. 1(D), the highest value of TN was observed in T4, ultrasonic process increase the VS and subsequently more organic compounds are consumed by microorganism and much nitrogen in forms of ammonia and N₂O are released depending on pH conditions^{38,39}. The fact of fluctuating trend in TN is that some microorganisms depending on temperature and pH are not able to do nitrification and denitrification. The increases in TN value are comparable with finding achieved by¹ when applied nitrogen source to improve the sewage sludge composting. According to Kruskal-Wallis statistical test, significant differences are observed among different reactors (P < 0.05).

C: *N* ratio. C: N ratio was calculated from the measured values of C and N. The C: N ratio of all reactors after a 35-day period of time were in range of 23.74–28.08. The lowest value belonged to T5, where a combination of ozonation and ultrasonics was applied to primary-stabilization of sludge. C:N ratio is an important indication for maturity of compost^{11,40} and the value below 20 is indicative of acceptable maturity of compost. The final C:N ratio of T3, T4, and T5 are lower than findings reported by other researchers⁶. However, these results are comparable with results obtained from³⁴ where applied successive composting and vermicomposting for biostabilization of tannery sludge mixed with cattle dung. According to Kruskal-Wallis statistical test, a significant difference was observed among different reactors (p-value < 0.05). The fluctuating trends of all reactors are shown in Fig. 1(E).

Total phosphorous (TP). Total phosphorous (TP) content of composting process were significantly increased as shown in Fig. 1(F). TP content of composting varied in range of 1.01-3.81%. The maximum increase was observed in T2, followed by T4, T5, T1, and T3. The increases in TP after composting process were supported by^{6,41}. Increased TP in composting process is assumed to be the results of increases in solubolization of organic matter and release phosphorous form these by microorganism present in composting process. According to Kruskal-Wallis statistical test, a significant difference was observed among different reactor (p-value < 0.05).

Microbial parameters. The microbial parameters in terms of total coliform (TC) and fecal coliform (FC) are presented in Table 2. The maximum and minimum reduction for TC were found to belong T3 (83%) and T1 (40%), respectively. As for FC reduction, the maximum and minimum reduction occurred in the reactors T5 (95%) and T1 (30%). According to values reported in Table 2, it can be concluded that T3, ozonation alone as primary-stabilizing method along with composting process is capable of significant reduction in all microbiological parameters. Therefore, this process can be a suitable alternative for pathogen reduction and ensure pathogen removal not safely occurred in even vermicomposting⁴². However, the simultaneous ozonation and ultrasonics integrated with composting process is the best option in terms of microbiological parameters for composting process so as to meet the standards for agricultural uses.

Conclusion

The integration ozonation and ultrasonic as primary-stabilizing methods along with composting as biological process is deliberated to be cost-effective and environmentally friendly for sludge stabilization before land and agronomic applications. A high degree of WAS stabilization was achieved in the study in terms of significant reduction in VS, TOC, TC, C/N ratio and pathogens along with the substantial increase in availability of nutrients like N and P. The efficiency of ozonation process for sludge pathogens removal was considerably high compared to ultrasonic process. However, ultrasonic process is a promising technology to increase organic matters and accordingly sludge dewaterability. The physicochemical characteristics of composting results including VS, TOC, TN, C/N, and TP are in range recommended for discharge into environment so that these parameters are comparable with resultant products from vermicomposting process. Therefore, composting system integrated with ultrasonic



Figure 2. Schematic reactors: Ozonation (**a**) and Ultrasonic (**b**) for sludge semi-stabilization (Designed by Adobe Illustrator software (Version 2019) https://www.adobe.com/products/illustrator.html.

and ozonation technologies as sludge primary-stabilization can potentially stabilizes and converts the hazardous WAS into non-hazardous nutrients-rich organic fertilizer and/or soil conditioner. It is important to note that the causes of variation of sludge characteristics following using ozonation and ultrasonic before and during composting process need further studies and consideration.

Methods and Materials

Waste activated sludge (WAS). Raw waste activated sludge was obtained on periodical basis whenever required from returned activated sludge line of municipal wastewater treatment plant (MWTT), Tehran, Iran. The fresh sludge with high moisture content (MC) was collected in large-sized plastic containers and then immediately transferred to the laboratory. In order to avoid any damage to high value of MC, the samples were stored at 4 °C until further processing. The physico-chemical and microbiological characteristics of raw WAS were analyzed according to the procedures outlined in the Standard Methods⁴³, as summarized in Table 1.

Green wastes. Green wastes (i.e. fruits and vegetables and etc.) were procured from wastes produced through domestic activities as bulking materials in the composting process. The composting material were cut into 2–3 cm length pieces. Physico-chemical and microbiological characteristics of green wastes are also reported in Table 1.

Ozonation/Ultrasonic for sludge primary-stabilization. Sludge ozonation was conducted in a 3-L volume batch reactor made of polymethyl methacrylate at temperature room)25 °C). Ozone was generated from pure oxygen by an ozone generator (Denali Pasargad, Iran). The oxygen gas flow rate was 1.5 L/min with an ozone mass concentration of approximately 6 g/L. Ozone was distributed into sludge sample by a microporous diffuser at the bottom of the reactor. Ozone consumption (mg/L) was calculated based on the amount of ozone feed to reactor minus amount leaving reactor. Amount of extra ozone leaving the reactor was measured by iodometric method. Figure 2(a) shows a schematic of ozonation reactor used in the present study. After ozonation, the physico-chemical and microbiological properties of resultant sludge were characterized, as presented in Table 1.

Floc deagglomeration experiment was performed using an ultrasonic homogenizer (Bandling sonoplus HD 2070, Germany) with an operating frequency of 20 kHz and power inputted to sludge was 120 W. The ultrasonic transducer was equipped with a titanium alloy horn with a 6-mm diameter. The ultrasonic irradiation time was 1 min, which was determined by the preliminary experiments. The schematic ultrasonic reactor is presented in Fig. 2(b). As well, the physico-chemical and microbiological properties of sludge after ultrasonic treatment are summarized in Table 1.

Experimental design. Five rectangular plastic containers with size of 0.3 m (length) $\times 0.3 \text{ m}$ (width) $\times 0.2 \text{ m}$ (height) were filled with a ratio of 2:1 green wastes to sludge (weight basis)⁹. Three kilograms of feed mixture was put in each rectangular plastic reactor. Composting experiments were established for 35 days and the sampling were prepared with a 7-day interval on days 0, 7,14,21,28 and 35. The zero day refers to the time of initial mixing of the sludge with different properties and green wastes before preliminary decomposition. The samples were air dried and ground in a stainless steel blender and stored in plastic vials for further chemical analysis. The experiments of each parameter was conducted in triplicate under laboratory conditions and the value reported on average. In order to maintain the temperature, eliminate volatile toxic substances and ensure aeration, each heap pile was turned over manually every 24h throughout the study period. Heap pile temperature was measured at two-third depth from heap surface. The pH was measured by a pH meter. The composition of sludge and green wastes in five different reactors are summarized in Table 3.

Physico-chemical and microbiological analysis. Compost were tested for evaluation the parameters affecting sludge stabilization. The samples were prepared on days 0, 7, 14, 21, 28, and 35 in triplicate and the

Treatment	Treatment Description
T ₁	Composting material (control)
T ₂	$Raw \ sludge + composting \ material$
T ₃	Ozonized sludge + composting material
T ₄	$Ultrasonic \ sludge + composting \ material$
T ₅	Combined ultrasonic/ Ozonized sludge + composting material

Table 3. Composition of Treatments.

-

values of parameters were reported on average. A sample of each treatment was collected and dried at 105 °C for 1 hour and then ground for estimation of various parameters except pH, moisture content (MC). The pH was measured using digital pH meter (HQ40d, HACH, USA) in 1/10 (w/v) of composting sample to deionized water. Moisture content was determined after drying the sample at 70 ± 2 °C for 24 h using hot air oven. Total solids (TS) were determined with the difference between before and after drying the sample at 105 °C. The VS content was determined by combusting the dried sample at 550 °C for 2 h in muffle furnace. Total organic carbon (TOC) was measured on dried samples using total organic carbon analyzer (TOC analyzer, multi N/C 3100, Germany). Total nitrogen was measured by micro Kjeldahl method⁴⁴. C:N ratio was calculated from the measured values of C and N. Total P was measured by acid digesting the samples and subsequently using ammonium molybdate method⁴³. The microbiological characterization of sample including total coliform (TC) and fecal coliform (FC) were analyzed according to Multiple-tube Fermentation Technique (9221 C and 9221E, standard methods of water and wastewater examination). All samples were measured in triplicate.

Statistical analysis. The data obtained from experiment were analyzed statistically using Minitab 16.0 software and GraphPad 5. The non-parametric statistical analysis Kruskal-Wallis was conducted at the 95% confidence level (significance was defined as P < 0.05) to analyze the significant difference between different experimental conditions (Parameters studied) in five reactors studied for different chemical parameters.

Received: 4 August 2019; Accepted: 8 January 2020; Published online: 23 January 2020

References

- 1. Meng, L. *et al.* Improving sewage sludge composting by addition of spent mushroom substrate and sucrose. *Bioresour. Technol.* 253, 197–203 (2018).
- 2. Gupta, R. & Garg, V. K. Stabilization of primary sewage sludge during vermicomposting. J. Hazard. Mater. 153, 1023-1030 (2008).
- 3. Liu, X. *et al.* Unveiling the mechanisms of how cationic polyacrylamide affects short-chain fatty acids accumulation during long-term anaerobic fermentation of waste activated sludge. *Water Res.* **155**, 142–151 (2019).
- 4. Villar, I., Alves, D., Pérez-Díaz, D. & Mato, S. Changes in microbial dynamics during vermicomposting of fresh and composted sewage sludge. *Waste Manag.* 48, 409–417 (2016).
- Cukjati, N., Zupančič, G. D., Roš, M. & Grilc, V. Composting of anaerobic sludge: An economically feasible element of a sustainable sewage sludge management. J. Environ. Manage. 106, 48–55 (2012).
- Suthar, S. & Singh, S. Feasibility of vermicomposting in biostabilization of sludge from a distillery industry. Sci. Total Environ. 394, 237–243 (2008).
- Elvira, C., Sampedro, L., Dominguez, J. & Mato, S. Vermicomposting of wastewater sludge from paper-pulp industry withnitrogen rich materials. Soil Biol. Biochem. 29, 759–762 (1997).
- 8. Domínguez, J., Edwards, C. A. & Webster, M. Vermicomposting of sewage sludge: Effect of bulking materials on the growth and reproduction of the earthworm Eisenia andrei. 32, 24–32 (2000).
- Hait, S. & Tare, V. Optimizing vermistabilization of waste activated sludge using vermicompost as bulking material. Waste Manag. 31, 502–511 (2011).
- Wu, C., Li, W., Wang, K. & Li, Y. Usage of pumice as bulking agent in sewage sludge composting. *Bioresour. Technol.* 190, 516–521 (2015).
- 11. Suthar, S., Mutiyar, P. K. & Singh, S. Vermicomposting of milk processing industry sludge spiked with plant wastes. *Bioresour. Technol.* **116**, 214–219 (2012).
- Wilson, C. A. & Novak, J. T. Hydrolysis of macromolecular components of primary and secondary wastewater sludge by thermal hydrolytic pretreatment. Water Res. 43, 4489–4498 (2009).
- 13. Kampas, P. *et al.* Mechanical sludge disintegration for the production of carbon source for biological nutrient removal. *Water Res.* **41**, 1734–1742 (2007).
- 14. Liu, X. *et al.* Freezing in the presence of nitrite pretreatment enhances hydrogen production from dark fermentation of waste activated sludge. *J. Clean. Prod.* 119305, https://doi.org/10.1016/j.jclepro.2019.119305 (2019).
- Guo, W. Q., Ding, J., Cao, G. L., Ren, N. Q. & Cui, F. Y. Treatability study of using low frequency ultrasonic pretreatment to augment continuous biohydrogen production. *Int. J. Hydrogen Energy* 36, 14180–14185 (2011).
- Nguyen, D. D. et al. Enhanced efficiency for better wastewater sludge hydrolysis conversion through ultrasonic hydrolytic pretreatment. J. Taiwan Inst. Chem. Eng. 71, 244–252 (2017).
- 17. Carbajo, J. B. *et al.* Ozonation as pre-treatment of activated sludge process of a wastewater containing benzalkonium chloride and NiO nanoparticles. *Chem. Eng. J.* **283**, 740–749 (2016).
- 18. Chu, L. B. et al. Enhanced sludge solubilization by microbubble ozonation. Chemosphere 72, 205-212 (2008).
- 19. Cheng, C. J., Andy Hong, P. K. & Lin, C. F. Improved solubilization of activated sludge by ozonation in pressure cycles. *Chemosphere* 87, 637–643 (2012).
- 20. Pei, J. et al. Effect of ultrasonic and ozone pre-treatments on pharmaceutical waste activated sludge's solubilisation, reduction, anaerobic biodegradability and acute biological toxicity. Bioresour. Technol. 192, 418–423 (2015).
- Zhao, F. & Cheng, D. Changes in pore size distribution inside sludge under various ultrasonic conditions. Ultrason. Sonochem. 38, 390–401 (2017).
- 22. Zhao, H., Zhang, P., Zhang, G. & Cheng, R. Enhancement of ultrasonic disintegration of sewage sludge by aeration. J. Environ. Sci. (China) 42, 163–167 (2016).

- Kavitha, S., Banu, J. R., Kumar, G., Kaliappan, S. & Tae Yeom, I. Profitable ultrasonic assisted microwave disintegration of sludge biomass: modelling of biomethanation and energy parameter analysis. *Bioresour. Technol.* https://doi.org/10.1016/j. biortech.2018.01.072 (2018).
- Cheng, C. J. & Hong, P. K. A. Anaerobic digestion of activated sludge after pressure-assisted ozonation. *Bioresour. Technol.* 142, 69–76 (2013).
- Zhang, G., Yang, J., Liu, H. & Zhang, J. Sludge ozonation: Disintegration, supernatant changes and mechanisms. *Bioresour. Technol.* 100, 1505–1509 (2009).
- 26. Laporte, A., Hawari, J., Barbeau, B. & Comeau, Y. Chemosphere Effect of ozonation on anaerobic digestion sludge activity and viability. 176, 405–411 (2017).
- Braguglia, C. M., Gianico, A. & Mininni, G. Comparison between ozone and ultrasound disintegration on sludge anaerobic digestion. J. Environ. Manage. 95, S139–S143 (2012).
- Zhang, J. et al. Changes of physicochemical properties of sewage sludge during ozonation treatment: Correlation to sludge dewaterability. Chem. Eng. J. 301, 238–248 (2016).
- 29. Cheng, C. *et al.* Enhancement of sludge reduction by ultrasonic pretreatment and packing carriers in the anaerobic side-stream reactor: Performance, sludge characteristics and microbial community structure. *Bioresour. Technol.* **249**, 298–306 (2018).
- Tian, X., Wang, C., Prandota, A., Lin, L. & Jern, W. Chemosphere Interpreting the synergistic effect in combined ultrasonication ozonation sewage sludge pre-treatment. *Chemosphere* 140, 63–71 (2015).
- Erden, G., Demir, O. & Filibeli, A. Disintegration of biological sludge: Effect of ozone oxidation and ultrasonic treatment on aerobic digestibility. *Bioresour. Technol.* 101, 8093–8098 (2010).
- 32. Chu, L. *et al.* Changes in biomass activity and characteristics of activated sludge exposed to low ozone dose. *Chemosphere* 77, 269–272 (2009).
- Christensen, E. & Giese, A. C. Changes in absorption spectra of nucleic acids and their derivatives following exposure to ozone and ultraviolet radiations. Arch. Biochem. Biophys. 51, 208–216 (1954).
- Vig, A. P., Singh, J., Wani, S. H. & Singh Dhaliwal, S. Vermicomposting of tannery sludge mixed with cattle dung into valuable manure using earthworm Eisenia fetida (Savigny). *Bioresour. Technol.* 102, 7941–7945 (2011).
- 35. Dominguez, J. & Edwards, C. A. Vermicomposting organic wastes: A review. Soil Zool. Sustain. Dev. 21st century (2004).
- Malińska, K., Zabochnicka-Światek, M., Cáceres, R. & Marfa, O. The effect of precomposted sewage sludge mixture amended with biochar on the growth and reproduction of Eisenia fetida during laboratory vermicomposting. *Ecol. Eng.* 90, 35–41 (2016).
- Meng, L., Li, W., Zhang, S., Wu, C. & Wang, K. Effects of sucrose amendment on ammonia assimilation during sewage sludge composting. *Bioresour. Technol.* 210, 160–166 (2016).
- Cáceres, R., Flotats, X. & Marfà, O. Changes in the chemical and physicochemical properties of the solid fraction of cattle slurry during composting using different aeration strategies. *Waste Manag.* https://doi.org/10.1016/j.wasman.2005.06.013 (2006).
- 39. Malińska, K. *et al.* Biochar amendment for integrated composting and vermicomposting of sewage sludge The effect of biochar on the activity of Eisenia fetida and the obtained vermicompost. *Bioresour. Technol.* **225**, 206–214 (2017).
- 40. Maňáková, B., Kuta, J., Svobodová, M. & Hofman, J. Effects of combined composting and vermicomposting of waste sludge on arsenic fate and bioavailability. *J. Hazard. Mater.* **280**, 544–551 (2014).
- Vandecasteele, B., Sinicco, T., D'Hose, T., Vanden Nest, T. & Mondini, C. Biochar amendment before or after composting affects compost quality and N losses, but not P plant uptake. J. Environ. Manage. 168, 200–209 (2016).
- Yadav, K. D., Tare, V. & Ahammed, M. M. Vermicomposting of source-separated human faeces for nutrient recycling. Waste Manag. 30, 50–56 (2010).
- 43. APHA/AWWA/WEF. Standard Methods for the Examination of Water and Wastewater. *Stand. Methods* 541. doi:ISBN 9780875532356 (2012).
- 44. Jackson, H. Chemical methods of male contraception. Am. Sci. 61, 188-193 (1973).

Acknowledgements

The authors gratefully acknowledge the financial support given by Research Center for Environmental Health Technology, Iran University of Medical Sciences, Tehran, Iran. (Grant Number 94-01-27-27754).

Author contributions

Mahdi Farzadkia and Ahmad Jonidi Jafari provided the idea for this work, Hamideh Dastpak and Hasan Pasalari designed the study and performed the experiments. Hamideh Dastpak, Hasan Pasalari, and Mitra Gholami contributed to data gathering, performed statistical data analysis, prepared all figures and tables, and wrote the main manuscript

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to M.F.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2020