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Direct resource recovery from sewage using a combined system of anaerobic-aerobic biological treatment and food production

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ABSTRACT

A combined system of an anaerobic baffled reactor (ABR), a down-flow hanging sponge (DHS) reactor, an aquarium tank (AT), and a constructed wetland (CWL) was proposed as a new concept for sewage treatment. The ABR and DHS reactor, AT, and CWL were applied for biological sewage treatment, bioassay, and nutrient removal with food production, respectively. Killifishes and tomatoes were cultivated in the AT and CWL, respectively. In the ABR, 81.3% of total chemical oxygen demand and 76.5% of total biochemical oxygen demand were removed at 5.1 h of hydraulic retention time (HRT). Most remaining organic matter and 47.1% of ammonia were removed in the DHS reactor. In the CWL, 97.0% of total inorganic nitrogen and 78.6% of phosphate were removed with 3.87 kg/m^2 of tomatoes producing yield at 4.4 days of HRT. In addition, anaerobic ammonium-oxidizing bacteria *Candidatus Scalindua* and ammonia-oxidizing bacteria *Nitrospira* and *Nitorosococcus* were considered as contributors to nitrogen removal in the CWL. The final effluent's water can be utilized as recycled water by installation of sand filtration and disinfection processes. Therefore, the proposed system can be applied as a low-energy, low-cost sewage treatment system with direct resource recovery.

Key words: constructed wetland, down-flow hanging sponge reactor, food chain, food production, sewage treatment

HIGHLIGHTS

- Anaerobic baffled reactor removed most of organic matters in sewage directly.
- Foods for killifishes were automatically fed by sewage treatment.
- Nutrients in sewage were consumed by tomatoes in constructed wetland.
- Proposed system achieved direct resource recovery from sewage.

INTRODUCTION

A sewage treatment system is a necessary infrastructure for improving public health. Activated sludge technology is a conventional sewage treatment process, which requires large consumption of energy, cost, and natural resources for both sewage and excess sludge treatment (Guo *et al.* 2013). In contrast, sewage can be also used to recover energy, nutrients, and water resources (Mo & Zhang 2013). Many studies on energy and resource recovery from sewage sludge have been conducted using anaerobic digestion, composting, and hydrothermal liquefaction (Wei *et al.* 2018; Du *et al.* 2019; Das *et al.* 2020). However, these technologies do not contribute to considerable reduction in power consumption and operating costs for sewage treatment. Recently, studies on direct recovery of energy and nutrients during sewage treatment have been conducted using anaerobic reactors, microbial fuel cells, algae, and aquatic plants (Liu *et al.* 2017; Nie *et al.* 2017; Aberysiriwardana-Arachchige *et al.* 2020; Iwano *et al.* 2020). Therefore, direct recovery of resources during sewage treatment can become essential for new-generation sewage treatment.

An anaerobic baffled reactor (ABR) and an up-flow anaerobic sludge blanket (UASB) reactor have been applied as anaerobic sewage treatment reactors in developing countries owing to their low cost of operation, construction, and maintenance (Yulistyorini *et al.* 2019; Passos *et al.* 2020). Trickling filters, which can be operated aerobically without aeration, were combined with these anaerobic reactors for sewage treatment (Bressani-Riveiro *et al.* 2019; Mazhar *et al.* 2021). In trickling filters, worms were grown and contributed to the reduction

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of excess sludge production in the food chain (Tamis *et al.* 2011). However, outbreak of worms becomes a sanitary problem during sewage treatment. In contrast, worms also become a natural feed for fishes when the system is combined with an aquarium tank (AT). In addition, fishes can be applied for bioassay (Berrebaan *et al.* 2020).

A constructed wetland (CWL) can be applied for direct nutrient recovery from sewage. In CWLs, nutrients in sewage can be harvested as food such as vegetables and fruits (Verhofstad *et al.* 2017). However, the nutrient removal efficiency of CWLs is still not as high in raw sewage treatment because of the high amount of organic matter (Jóźwiakowski *et al.* 2020).

Therefore, a new concept of sewage treatment based on the combination of biological treatment processes and food production was proposed in this study. The performance of the sewage treatment system, removal efficiency of organic matters, nitrogen and phosphate, methane recovery in the anaerobic reactor, food chain in the AT, food production in the CWL, and water quality of the final effluent were evaluated.

MATERIALS AND METHODS

Experimental setup

Figure 1 shows a schematic diagram of the sewage treatment system used in this study. The sewage treatment system comprises an ABR, a DHS reactor, an AT, and a CWL. In the proposed system, organic matter in sewage was removed in the ABR–DHS system. Fishes in the AT were grown by consuming metazoans in the DHS effluent. Nutrients in sewage were recovered as vegetables in the CWL. The final effluent was considered as recycled water in the sewage treatment plant.

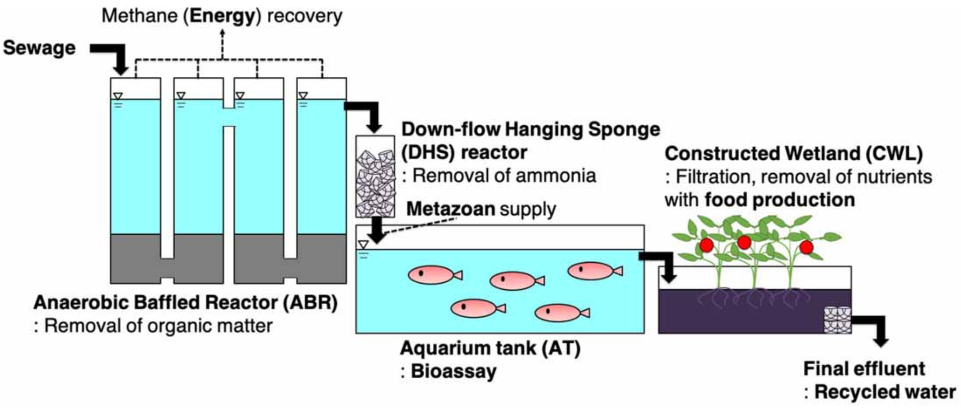

Figure 1 | Schematic diagram of the sewage treatment system.

The ABR was manufactured of polyvinyl chloride pipes (diameter: 30 mm). The ABR includes four compartments with an effective volume of 0.8 L. The DHS reactor was manufactured of a polyethylene terephthalate bottle (volume: 0.8 L) packed with polyurethane sponges as a biomass carrier. The AT and CWL were manufactured from 20 L plastic tanks. Killifishes (*Oryzias*) were bred in the AT. Tomatoes (*Lycopersicum esculentum*) were cultivated in the CWL. The landslide of the heavy rain disaster in Hiroshima was utilized as soil for the CWL. Polyurethane sponges were installed near the effluent of the CWL to prevent soil discharge. The granular sludge of the UASB reactor treating food processing wastewater was seeded in the ABR. The DHS reactor was started up without any seed sludge. The system was operated in a temperature-controlled room at 25 °C.

Synthetic sewage was obtained by mixing soy protein (as proteins), waste rice wine (as carbohydrates), waste oil (as lipids), toilet paper (as cellulose), and waste miso (as minerals) using a similar content ratio of carbohydrates, proteins, lipids, and cellulose in the sewage. The synthetic sewage had a total chemical oxygen demand (COD) of 364 ± 93 mg/L, total biochemical oxygen demand (BOD) of 364 ± 142 mg/L, ammonia of 21 ± 8 mgN/L, phosphate of 1.1 ± 0.6 mgP/L, and pH of 6.48 ± 0.82. The flow rate of synthetic sewage incrementally increased from 1.9 to 3.7 L/day.

Chemical analysis

Wastewater samples were collected twice weekly from the sewage tank, ABR, DHS, and final effluents. The pH value was measured using a pH meter (Twin pH, Horiba, Japan). Total COD and total nitrogen were analyzed by a Hach apparatus (DR/2500, Hach, USA). Ammonia, nitrite, and nitrate concentrations were measured using an ion chromatograph equipped with a conductivity detector (CDD-10A VP, Shimadzu, Japan). Phosphate, color, and turbidity were analyzed by a digital packtest (DPM-MTSP, Kyoritsu Chemical-Check Lab., Japan). Methane concentrations in ABR biogas were analyzed using a gas chromatograph (GC-8A, Shimadzu, Japan) equipped with a thermal conductivity detector. BOD was analyzed using a standard method (APHA 2012). Coliforms were measured using petrifilm (6404EC, 3M company, USA).

Microbial community analysis

The analysis of microbial communities in DHS effluents and the AT (18S rRNA gene), and in the DHS reactor and final effluent (16S rRNA gene) was conducted. DNA extraction was performed using a MonoFas Bacterial Genomic Kit VII (Animos Corporation, Saitama, Japan). Polymerase chain reaction (PCR) amplification of the 18S rRNA gene V4 and V9 region (Hirakata *et al.* 2019) and 16S rRNA gene V3–V4 region (Caporaso *et al.* 2012) was performed using a Sapphire Amp Fast PCR Master Mix (Takara, Shiga, Japan). PCR products were purified using AMPure XP SPRI beads (Beckman). Massive parallel 16S rRNA gene sequencing and functional genes were conducted by the Miseq System (Illumina) using a Miseq Reagent Kit v.3 (2x 300 bp). Taxonomic classification and read count metrics were determined using the 16S Metagenomics Application v.1.0.0.1024. The algorithm used in this program is a high-performance implementation of the Ribosomal Database Project where reads reference an Illumina-curated version of the GreenGenes (May 2013) taxonomic database (Wang *et al.* 2007) for 16S rRNA gene, and QIIME 2 View for 18S rRNA gene (Bolyen *et al.* 2019).

RESULTS AND DISCUSSION

Sewage treatment performance of the ABR–DHS system

Figure 2 shows the average COD and BOD concentrations of the influent, ABR, and DHS effluents. In the ABR, 81.3 ± 12.5% of total COD and 76.5 ± 14.6% of total BOD were removed. Finally, 91.0 ± 5.5% of total COD and 85.5 ± 12.4% of total BOD were removed by the ABR–DHS system. During the experimental period, the HRT of the ABR–DHS system was reduced from 12.2 to 6.1 h. This process performance was similar to that of activated sludge processes (Nakada *et al.* 2006; Tandukar *et al.* 2007), ABRs (Krishna *et al.* 2008; Hahn & Figueroa 2015), and combination systems of anaerobic–aerobic reactors (Nomoto *et al.* 2018; Mazhar *et al.* 2021) treating sewage. In the ABR, 0.02 m^3/m^3-sewage of biogas was produced at 5.1 h of HRT. By anaerobic digestion of excess sewage sludge, 0.5 m^3/m^3-sewage of biogas can be recovered (Cao & Pawtowski 2012; Jenicek *et al.* 2012; Li *et al.* 2013). Methane recovery of the ABR is only 4% of anaerobic digestion of excess sewage sludge. However, the ABR–DHS system can be operated without power consumption for aeration and excess sludge production. Therefore, the ABR–DHS system mainly contributes to reduction of power consumption for sewage treatment, not for energy recovery from sewage treatment.

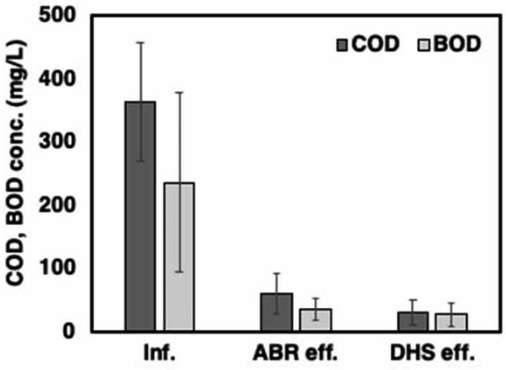

Figure 2 | Average COD and BOD concentrations of the influent, ABR, and DHS effluents.

Figure 3 shows the average ammonia and nitrate concentrations of the ABR and DHS effluents. Nitrite was not confirmed in the DHS effluent during the entire experimental period. In the DHS reactor, 47.1 ± 27.0% of ammonia was removed, and 56.8 ± 26.9% of removed ammonia remained in the effluent as nitrate. Nitrogen

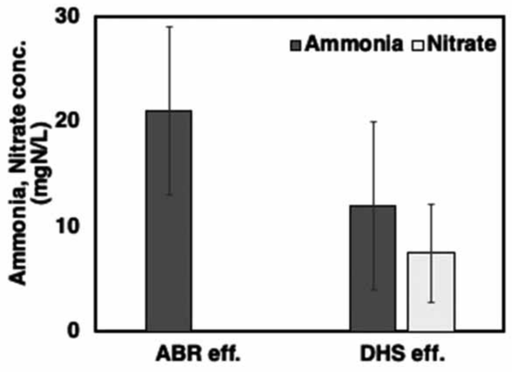

Figure 3 | Average ammonia and nitrate concentrations of the ABR and DHS effluents.

removal efficiencies of DHS reactors treating sewage were in the range of 30–80% (Tawfik *et al.* 2006; Tandukar *et al.* 2007; Onodera *et al.* 2014). Compared with that in these previous studies, nitrogen removal efficiency of the DHS reactor in this study was lower. However, dissolved oxygen concentration in the AT was kept at 5.74 mg/L on average without aeration at 4.6–9.2 days of the HRT. In addition, killifishes were grown without any feed, and eutrophication was not confirmed in the AT during the entire experimental period. Therefore, the water quality of the DHS effluent was acceptable for autopurification and contributed to the food chain in the AT.

Food chain in AT

Figure 4 shows predominant eukaryotic microorganisms in the DHS effluent and AT. The yield of 18S rRNA PCR product amplified from DNA extracted from the DHS effluent and AT was 12 ng/μL and 0.2 ng/μL, respectively. In the DHS effluent, *Chlorophyta* (predominant species: *Desmodesmus cf. armatus*), *Apicomplexa*, *Cecozoa*, *Nematoda*, and *Rotifera* (predominant species: *Habrotrocha bidens*) were the predominant phyla. Among these microorganisms, *Nematoda*, *Apicomplexa*, and *Rotifera* were highly reduced in the AT. In addition, *Ciliphora* (predominant species: *Hypotrichidium paraconicum*) and *Gastrotricha* (predominant species: *Chaetonotus acanthodes*) were increased in the AT. *Nematoda* and *Rotifera* were detected as major metazoans in the DHS reactor treating sewage and considered as contributors of excess sludge reduction (Onodera *et al.* 2013; Miyaoka *et al.* 2016). In contrast, *Ciliphora* and *Gastrotricha* were detected in the oxidation ditch process treating sewage, which has a longer HRT than have the DHS reactor and activated sludge process (Matsunaga *et al.* 2014). Furthermore, *Gastrotricha* increased in the sewage treatment process without detection of or with lower detection rate of *Nematoda* and *Rotifera* (Matsunaga *et al.* 2014; Miyaoka *et al.* 2016). In these metazoans, *Gastrotricha* has the smallest size (Segers 2008; Blaxter & Koutsovoulos 2015; Todaro *et al.* 2019). During the entire experimental period, worms were confirmed only in the DHS reactor, not in the AT. Therefore, *Nematoda*,

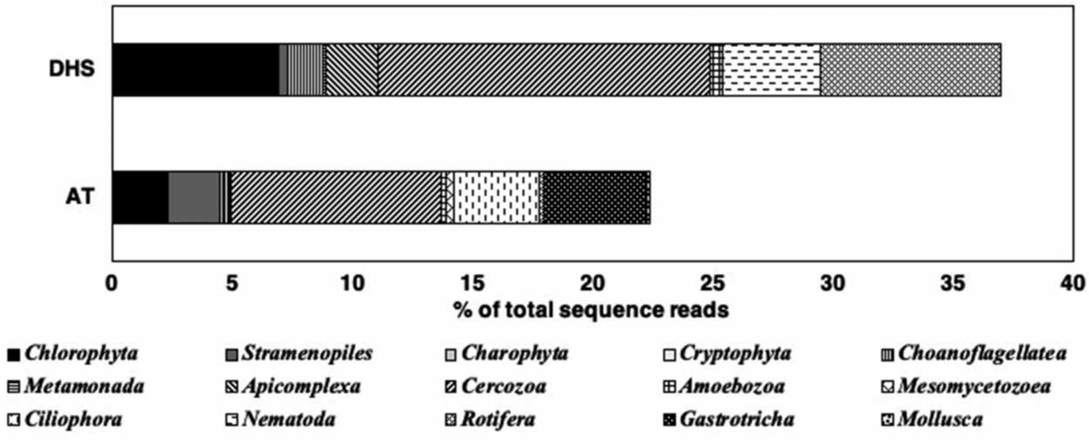

Figure 4 | Predominant eukaryotic microorganisms in the DHS effluent and AT.

Rotifera, and worms were considered as main foods for killifishes in the AT. Furthermore, an outbreak of worms was also prevented by combining the DHS reactor and the AT.

Nutrient removal and food production in the CWL

Figure 5 shows average total inorganic nitrogen (TIN) and phosphate concentrations of the DHS and final effluents. In the CWL, 97.0 ± 4.9% of TIN and 78.6 ± 6.9% of phosphate were removed at 4.4 days of HRT. Benvenuti *et al.* (2018) have applied a real-scale constructed floating wetland using *Typha domingensis* for domestic sewage treatment and achieved 41% of nitrogen removal and 37% of phosphorous removal at 11.5 days of the HRT. Ye *et al.* (2012) have applied real-scale ABR stage-CWL using *Phragmites autralis*, *Canna indica*, and *Cyperus alternifolius* and achieved 31% of nitrogen removal and 34% of phosphorous removal at 5.2 days of the HRT. Compared with the results of these studies, both nitrogen and phosphorous removal efficiencies in this study were much higher. The production yield of tomatoes in this study was 3.87 kg/m^2. This value was approximately five times higher than that obtained in a pilot-scale CWL treating domestic wastewater (0.73 kg/m^2; Caselles-Osorio *et al.* 2018). Furthermore, the obtained value was similar to the conventional production yield (5–60 kg/m^2) for organic tomatoes (Bosona & Gebresenbet 2018; Liang *et al.* 2019; Ronga *et al.* 2019). Nitrogen and phosphate contents of tomato fruits were 892 mgN/kg-fruits and 392 mgP/kg-fruits, respectively. In terms of mass balance, 3.0% of input nitrogen and 5.1% of input phosphate were harvested as tomato fruits. In addition, 4.0% of input nitrogen and 1.4% of input phosphate were accumulated as sludge at the bottom of the AT. As the results of microbial community analysis (yield of 16S rRNA PCR product: 5.1 ng/μL), anaerobic ammonium-oxidizing bacteria *Candidatus Scalindua* (1.0%) and ammonia-oxidizing bacteria *Nitrospira* (0.6%) and *Nitorosococcus* (0.2%) were detected in the final effluent (detection rates of total sequence reads). In this study, most organic matter was degraded in the ABR–DHS system; then, the CWL was mainly used for nutrient removal. Therefore, in this study, low organic loading rate contributed to high nutrient removal efficiency by both microbes and cultivation of tomatoes in CWL.

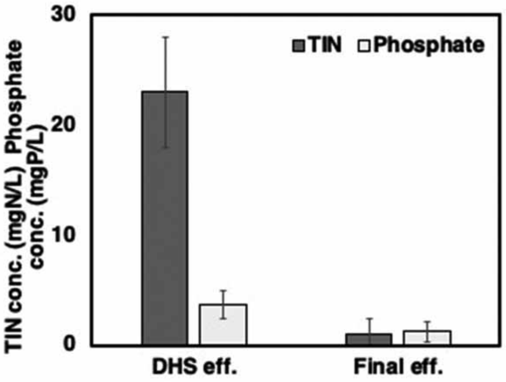

Figure 5 | Average TIN and phosphate concentrations of the DHS and final effluents.

Applicability of the final effluent for water recycling

Table 1 shows the water quality of the final effluent and Japanese quality standards for discharge and recycled water. The final effluent achieved the Japanese quality standard for discharge. However, only pH and odor parameters achieved the Japanese quality standard for recycled water. The color of the final effluent was the color of organic matter (e.g., soy protein and miso) in synthetic sewage. The coliform count and turbidity of the final effluent had values similar to those of the secondary effluent of sewage treatment plants (Uemura *et al.* 2002; Qin *et al.* 2006). Figure 6 shows time course of turbidity and coliform of final effluent. In terms of analytical results, 80% of coliform count was below 5 CFU/mL, and 40% of turbidity was below 2. Thus, sporadically high values highly affected the average value of these parameters. The results of microbial community analysis showed that some pathogenic bacteria, such as *Oligella ureolytica* and *Rickettsia* (Weinert *et al.* 2009; Demir & Celenk 2014), were also detected in approximately 1% of total sequence reads in the final effluent. Therefore, post-treatment processes (e.g., sand filtration and disinfection) were required before using the final effluent as recycled water.

Table 1 | Water quality of the final effluent and Japanese quality standards

			Japanese quality standard	
Parameters	**Unit**	**Final effluent**	**Discharge**	**Recycled water**
pH	–	7.0 ± 0.4	5.8–8.6	5.8–8.6
BOD	mg/L	25 ± 20	160	–
COD	mg/L	31 ± 19	160	–
Total nitrogen	mg/L	0.8 ± 1.4	120	–
Total phosphorous	mg/L	1.22 ± 0.88	16	–
Odor	–	Not aberrant	–	Not aberrant
Color	CU	193 ± 143	–	Almost clear and colorless
Coliform	CFU/mL	57 ± 124	3,000	Not detected
Turbidity	FTU	15 ± 21	–	Below 2

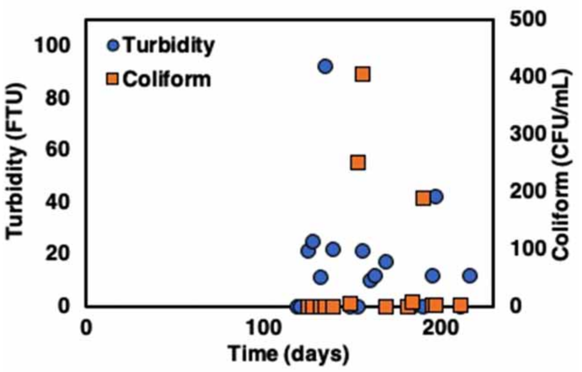

Figure 6 | Time course of turbidity and coliform of final effluent.

CONCLUSIONS

The results of this study indicated the high potential of the proposed sewage treatment system for pollutant removal and resource recovery from sewage. The ABR–DHS system achieved high performance of organic matter removal with biogas recovery at an HRT similar to that of activated sludge processes with a much lower power consumption. The water quality of DHS effluents was adequate for both autopurification and the food chain in the AT. In addition, the AT was used for bioassay and removal of worms, which occur in the DHS reactor. The CWL contributed to nutrient removal, food production, and water purification. The final effluent achieved the Japanese quality standards for discharge. However, additional post-treatments for removal of turbidity and pathogens, such as sand filtration and disinfection, were required to use the final effluent as recycled water.

The results of this study indicated an applicability of the proposed system for low-energy and low-cost sewage treatment with direct resource recovery. Furthermore, the proposed system can be applied by renovation of present sewage treatment systems such as activated sludge process. For practical use of the proposed system, some problems such as operation and maintenance of the system, climate property of the area, adaptable species for AT and CWL, and economical advantage, should be considered. As the next step, a pilot-scale experiment will be conducted to clarify these problems in future work.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Aberysiriwardana-Arachchige, I. S. A., Muanasinghe-Arachchige, S. P., Delanka-Pedige, H. M. K. & Nirmalakhandan, N. 2020 Removal and recovery of nutrients from municipal sewage: algal vs. conventional approaches. *Water Research* **175**, in press. https://doi.org/10.1016/j.watres.2020.115709.

APHA/AWWA/WEF 2012 *Standard Methods for the Examination of Water and Wastewater*, 22nd edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Benvenuti, T., Hamerski, F., Giacobbo, A., Bernardes, A. M., Zoppas-Ferreira, J. & Rodrigues, M. A. S. 2018 Constructed floating wetland for the treatment of domestic sewage: a real-scale study. *Journal of Environmental Chemical Engineering* **6**(5), 5706–5711. https://doi.org/10.1016/j.jece.2018.08.067.

Berrebaan, I., Montassir, L. & Bessi, H. 2020 Ecotoxicological bioassays: an innovative tool for wastewater pollution control. *Research Journal of Chemistry and Environment* **24**(2), 139–147.

Blaxter, M. & Koutsovoulos, G. 2015 The evolution of parasitism in *Nematoda*. *Parasitology* **142**, S26–S39. https://doi.org/S0031182014000791.

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K., Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M., Chase, J., Cope, E. K., Silva, R. D., Diener, C., Dorrestein, P. C., Douglas, G. M., Durall, D. M., Duvallet, C., Edwardson, C. F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J. M., Gibbons, S. M., Gibson, D. L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G. A., Janssen, S., Jarmusch, A. K., Jiang, L., Kaehler, B. D., Kang, K. B., Keefe, C. R., Keim, P., Kelley, S. T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M. G. I., Lee, J., Ley, R., Liu, Y.-X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B. D., McDonald, D., McIver, L. J., Melnik, A. V., Metcalf, J. L., Morgan, S. C., Morton, J. T., Naimey, A. T., Navas-Molina, J. A., Nothias, L. F., Orchanian, S. B., Pearson, T., Peoples, S. L., Petras, D., Preuss, M. L., Pruesse, E., Rasmussen, L. B., Rivers, A., Robeson II, M. S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A., Sinha, R., Song, S. J., Spear, J. R., Swafford, A. D., Thompson, L. R., Torres, P. J., Trinh, P., Tripathi, A., Turnbaugh, P. J., Ul-Hasan, S., van der Hooft, J. J. J., Vargas, F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang, M., Warren, J., Weber, K. C., Williamson, C. H. D., Willis, A. D., Xu, Z. Z., Zaneveld, J. R., Zhang, Y., Zhu, Q., Knight, R. & Caporaso, J. G. 2019 Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nature Biotechnology* **37**(8), 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Bosona, T. & Gebresenbet, G. 2018 Life cycle analysis of organic tomato production and supply in Sweden. *Journal of Cleaner Production* **196**, 635–643. https://doi.org/10.1016/j.jclepro.2018.06.087.

Cao, Y. & Pawtowski, A. 2012 Sewage sludge-to-energy approaches based on anaerobic digestion and pyrolysis: brief overview and energy efficiency assessment. *Renewable & Sustainable Energy Reviews* **16**(3), 1657–1665. https://doi.org/10.1016/j.rser.2011.12.014.

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G. & Knight, R. 2012 Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal* **6**, 1621–1624. https://doi.org/10.1038/ismej.2012.8.

Caselles-Osorio, A., Mendoza, G., Simanca, M., Romero-Borja, I., Mosquera, J. E. & Eslaba, P. 2018 Tomato (*Lycopersicum sculentum*) production in sub surface flow constructed wetlands for domestic wastewater treatment in rural a Colombian community. *Ingeniería Investigación y Technología* **19**(4), 1–10. http://dx.doi.org/10.22201/fi.25940732e.2018.19n4.038.

Das, P., Khan, S., Quadir, M. A., Thaher, M., Waqas, M., Easa, A., Attia, E. S. M. & Al-Jabri, H. 2020 Energy recovery and nutrients recycling from municipal sewage sludge. *Science of The Total Environment* **715**, in press. https://doi.org/10.1016/j.scitotenv.2020.136775.

Demir, T. & Celenk, N. 2014 Bloodstream infection with *Oligella ureolytica* in a newborn infant: a case report and review of the literature. *The Journal of Infection in Developing Countries* **8**, 793–795. https://doi.org/10.3855/jidc.3260.

Du, J., Zhang, Y., Qu, M., Yin, Y., Fan, K., Hu, B., Zhang, H., Wei, M. & Ma, C. 2019 Effects of biochar on the microbial activity and community structure during sewage sludge composting. *Bioresource Technology* **272**, 171–179. https://doi.org/10.1016/j.biortech.2018.10.020.

Guo, W.-Q., Yang, S.-S., Xiang, W.-S., Wang, X.-J. & Ren, N.-Q. 2013 Minimization of excess sludge production by in-situ activated sludge treatment processes – a comprehensive review. *Biotechnology Advances* **31**(8), 1386–1396. https://doi.org/10.1016/j.biotechadv.2013.06.003.

Hahn, M. & Figueroa, L. 2015 Pilot scale application of anaerobic baffled reactor for biologically enhanced primary treatment of raw municipal wastewater. *Water Research* **87**(15), 494–502. https://doi.org/10.1016/j.watres.2015.09.027.

Hirakata, Y., Hatamoto, M., Oshiki, M., Watari, T., Kuroda, K., Araki, N. & Yamaguchi, T. 2019 Temporal variation of eukaryotic community structures in UASB reactor treating domestic sewage as revealed by 18S rRNA gene sequencing. *Scientific Reports* **9**, 12783. https://doi.org/10.1038/s41598-019-49290-y.

Iwano, H., Hatohara, S., Tagawa, T., Tamaki, H. & Kubota, K. 2020 Effect of treated sewage characteristics on duckweed biomass production and microbial communities. *Water Science & Technology* **82**(2), 292–302. https://doi.org/10.2166/wst.2020.168.

Jenicek, P., Bartacek, J., Kutil, J., Zabranska, J. & Dohanyos, M. 2012 Potentials and limits of anaerobic digestion of sewage sludge: energy self-sufficient municipal wastewater treatment plant? *Water Science & Technology* **66**(6), 1277–1281. https://doi.org/10.2166/wst.2012.317.

Jóźwiakowski, K., Bugajski, P., Kurek, K., Cáceres, R., Siwiec, T., Juncherski, A., Czekałaf, W. & Kozłowskif, K. 2020 Technological reliability of pollutant removal in different seasons in one-stage constructed wetland system with horizontal flow operating in the moderate climate. *Separation & Purification Technology* **238**, 116439. https://doi.org/10.1016/j.seppur.2019.116439.

Krishna, G. V. T. G., Kumar, P. & Kumar, P. 2008 Treatment of low strength complex wastewater using an anaerobic baffled reactor (ABR). *Bioresource Technology* **99**(17), 8193–8200. https://doi.org/10.1016/j.biortech.2008.03.016.

Li, X., Takaoka, M., Zhu, F., Wang, J., Oshita, K. & Mizuno, T. 2013 Environmental and economic assessment of municipal sewage sludge management – a case study in Beijing, China. *Water Science & Technology* **67**(7), 1465–1473. https://doi.org/10.2166/wst.2013.688.

Liang, L., Ridoutt, B. G., Lal, R., Wang, D., Wu, W., Peng, P., Hang, S., Wang, L. & Zhao, G. 2019 Nitrogen footprint and nitrogen use efficiency of greenhouse tomato production in North China. *Journal of Cleaner Production* **208**, 285–296. https://doi.org/10.1016/j.jclepro.2018.10.149.

Liu, R., Tursun, H., Hou, X., Odey, F., Li, Y., Wang, X. & Xie, T. 2017 Microbial community dynamics in a pilot-scale MFC-AA/O system treating domestic sewage. *Bioresource Technology* **241**, 439–447. https://doi.org/10.1016/j.biortech.2017.05.122.

Matsunaga, K., Kubota, K. & Harada, H. 2014 Molecular diversity of Eukaryotes in municipal wastewater treatment process as revealed by 18S rRNA gene analysis. *Microbes & Environments* **29**(4), 401–407. https://doi.org/10.1264/jsme2.ME14112.

Mazhar, M. A., Khan, N. A., Khan, A. H., Ahmed, S., Siddiqui, A. A., Husain, A., Rahisuddin Tirth, V., Islam, S., Shukla, N. K., Changani, F., Yousefi, M., Hassaballa, A. E. & Radwan, N. 2021 Upgrading combined anaerobic-aerobic UASB-FPU to UASB-DHS system: cost comparison and performance perspective for developing countries. *Journal of Cleaner Production* **284**, in press. https://doi.org/10.1016/j.jclepro.2020.124723.

Miyaoka, Y., Hatamoto, M., Yamaguchi, T. & Syutsubo, K. 2016 Eukaryotic community shift in response to organic loading rate of an anaerobic trickling filter (down-flow hanging sponge reactor) treating domestic sewage. *Microbial Ecology* **73**(4), 801–814. https://doi.org/10.1007/s00248-016-0871-0.

Mo, W. & Zhang, Q. 2013 Energy–nutrients–water nexus: integrated resource recovery in municipal wastewater treatment plants. *Journal of Environmental Management* **127**, 255–267. https://doi.org/10.1016/j.jenvman.2013.05.007.

Nakada, N., Tanishima, T., Shinohara, H., Kiri, K. & Takada, H. 2006 Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. *Water Research* **40**(17), 3297–3303. https://doi.org/10.1016/j.watres.2006.06.039.

Nie, Y., Chen, R., Tian, X. & Li, Y.-Y. 2017 Impact of water characteristics on the bioenergy recovery from sewage treatment by anaerobic membrane bioreactor via a comprehensive study on the response of microbial community and methanogenic activity. *Energy* **139**, 459–467. https://doi.org/10.1016/j.energy.2017.07.168.

Nomoto, N., Hatamoto, M., Ali, M., Jayaswal, K., Iguchi, A., Okubo, T., Takahashi, M., Kubota, K., Tagawa, T., Uemura, S., Yamaguchi, T. & Harada, H. 2018 Characterization of sludge properties for sewage treatment in a practical-scale down-flow hanging sponge reactor: oxygen consumption and removal of organic matter, ammonium, and sulfur. *Water Science & Technology* **77**(3), 608–616. https://doi.org/10.2166/wst.2017.557.

Onodera, T., Matsunaga, K., Kubota, K., Taniguchi, R., Harada, H., Syutsubo, K., Okubo, T., Uemura, S., Araki, N., Yamada, M., Yamauchi, M. & Yamaguchi, T. 2013 Characterization of the retained sludge in a down-flow hanging sponge (DHS) reactor with emphasis on its low excess sludge production. *Bioresource Technology* **136**, 169–175. https://doi.org/10.1016/j.biortech.2013.02.096.

Onodera, T., Tandukar, M., Sugiyana, D., Uemura, S., Ohashi, A. & Harada, H. 2014 Development of a sixth-generation down-flow hanging sponge (DHS) reactor using rigid sponge media for post-treatment of UASB treating municipal sewage. *Bioresource Technology* **152**, 93–100. https://doi.org/10.1016/j.biortech.2013.10.106.

Passos, F., Bressani-Riveiro, T., Rezende, S. & Chernicharo, C. A. L. 2020 Potential application of biogas produced in small-scale UASB-based sewage treatment plants in Brazil. *Energies* **13**(13), 3356. https://doi.org/10.3390/en13133356.

Qin, J.-J., Wai, M. N., Oo, M. H., Kekre, K. A. & Seah, H. 2006 Feasibility study for reclamation of a secondary treated sewage effluent mainly from industrial sources using a dual membrane process. *Separation & Purification Technology* **50**(3), 380–387. https://doi.org/10.1016/j.seppur.2005.12.013.

Ronga, D., Gallingani, T., Zaccardelli, M., Perrone, D., Francia, E. & Pecchioni, N. 2019 Carbon footprint and energetic analysis of tomato production in the organic vs the conventional cropping systems in Southern Italy. *Journal of Cleaner Production* **220**, 836–845. https://doi.org/10.1016/j.jclepro.2019.02.111.

Segers, H. 2008 Global diversity of rotifers (*Rotifera*) in freshwater. *Hydrobiologia* **595**, 49–59. https://doi.org/10.1007/s10750-007-9003-7.

Tamis, J., van Schouwenburg, G., Kleerebezem, R. & van Loosdrecht, M. C. M. 2011 A full scale worm reactor for efficient sludge reduction by predation in a wastewater treatment plant. *Water Research* **45**(18), 5916–5924. https://doi.org/10.1016/j.watres.2011.08.046.

Tandukar, M., Ohashi, A. & Harada, H. 2007 Performance comparison of a pilot-scale UASB and DHS system and activated sludge process for the treatment of municipal wastewater. *Water Research* **41**(12), 2697–2705. https://doi.org/10.1016/j.watres.2007.02.027.

Tawfik, A., Ohashi, A. & Harada, H. 2006 Sewage treatment in a combined up-flow anaerobic sludge blanket (UASB)–down-flow hanging sponge (DHS) system. *Biochemical Engineering Journal* **29**, 210–219.

Todaro, M. A., Sibaja-Cordero, J. A., Segura-Bermúdez, O. A., Coto-Delgado, G., Goebel-Otárola, N., Barquero, J. D., Cullell-Delgado &, M. & Zotto, M. D. 2019 An introduction to the study of *Gastrotricha*, with a taxonomic key to families and genera of the group. *Diversity* **11**(117), 1–25.

Uemura, S., Takahashi, K., Machdar, I., Ohashi, A. & Harada, H. 2002 Removal of indigenous coliphages and fecal coliforms by a novel sewage treatment system consisting of UASB and DHS units. *Water Science & Technology* **46**(11–12), 303–309. https://doi.org/10.2166/wst.2002.0754.

Verhofstad, M. J. J. M., Poelen, M. D. M., van Kempen, M. M. L., Bakker, E. S. & Smolders, A. J. P. 2017 Finding the harvesting frequency to maximize nutrient removal in a constructed wetland dominated by submerged aquatic plants. *Ecological Engineering* **106**, 423–430. https://doi.org/10.1016/j.ecoleng.2017.06.012.

Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. 2007 Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**(16), 5261–5267. https://doi.org/10.1128/AEM.00062-07.

Wei, J., Hao, X., can Loosdrecht, M. C. M. & Li, J. 2018 Feasibility analysis of anaerobic digestion of excess sludge enhanced by iron: a review. *Renewable & Sustainable Energy Reviews* **89**, 16–26. https://doi.org/10.1016/j.rser.2018.02.042.

Weinert, L. A., Werren, J. H., Aebi, A., Stone, G. N. & Jiggins, F. M. 2009 Evolution and diversity of *Rickettsia* bacteria. *BMC Biology* **7**, 6. https://doi.org/10.1186/1741-7007-7-6.

Ye, C., Li, L., Zhan, J. & Yang, Y. 2012 Study on ABR stage-constructed wetland integrated system in treatment of rural sewage. *Procedia Environmental Sciences* **12**, 687–692. https://doi.org/10.1016/j.proenv.2012.01.335.

Yulistyorini, A., Camargo-Valero, M. A., Sukarni, S., Suryoputro, N., Mujiyono, M., Santoso, H. & Rahayu, E. T. 2019 Performance of anaerobic baffled reactor for decentralized wastewater treatment in urban Malang, Indonesia. *Processes* **7**(4), 184. https://doi.org/10.3390/pr7040184.

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