frontiers | Frontiers in Environmental Science

TYPE Review
PUBLISHED 07 December 2022
DOI 10.3389/fenvs.2022.1056019

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Water and Wastewater Management, a section of the journal Frontiers in Environmental Science

RECEIVED 28 September 2022
ACCEPTED 28 November 2022
PUBLISHED 07 December 2022

CITATION
Xu K, Lu J, Hu L, Li J, Cheng S, Zheng M and Wang C (2022), Pathogens inactivation in nutrient recovery from urine: A review.
Front. Environ. Sci. 10:1056019.
doi: 10.3389/fenvs.2022.1056019

Pathogens inactivation in nutrient recovery from urine: A review

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Urine source separation, a kind of new sewage management concept, has made great progress in technology development and application in the past 30 years. However, understanding of the potential microbial risks in reuse of urine-derived fertilizer products (UDFPs) in agriculture is still lacking. Outbreak of pandemic of Coronavirus Disease 2019 and more deadly disease caused by Monkeypox strongly sounds the alarm bell to the attention on pathogens in urine and their fate in UDFPs. Therefore, this study presented a comprehensive review on pathogens inactivation in nutrient recovery technologies. The review suggests that technologies using alkaline or heating treatment can effectively reduce pathogens in UDFPs. However, technologies with characteristics such as membrane rejection of nutrients or nutrient adsorption may even concentrate pathogens in their fertilizer products. Based on an overall assessment, connections of technologies and the pathogens inactivation in their UDFPs have been established. This would help to provide a perspective on development of urine treatment technology and management of microbial risks in reusing urine nutrients in agriculture.

KEYWORDS

urine source separation, nutrient recovery, pathogens inactivation, microbial risk, urine treatment

1 Introduction

Urine source separation, a novel concept of wastewater management, was proposed at the end of the 19th century (Larsen and Gujer, 1996). Human urine contains abundant amounts of macro-nutrients, accounting for approximately 79% of nitrogen, 49% of phosphorus, and 58% of potassium in household wastewater (Vinnerås et al., 2006). Resource recycling from urine benefits closing the loop of nutrients. This would provide a sustainable approach to ease the tension between the increasing demand of food and fertilizer and the shortage of nutrients while preventing water pollution (Larsen et al., 2016; Badeti et al., 2022). Thus, it could be an effective tool to help eradicating hunger and ensuring environmental sustainability in the Millennium Development Goals (MDGs) of

United Nations, and achieving zero hunger, clean water and sanitation, and sustainable cities and communities in the Sustainable Development Goals (SDGs).

Various technologies have been developed to reuse or recover nutrients from urine. This has been reviewed in several recent publications (Simha and Ganesapillai, 2017; Harder et al., 2019; Patel et al., 2020; Cheng et al., 2022; Liu et al., 2022). Moreover, dozens of pilot projects of urine source separation have been constructed and evaluated in schools, villages or modern buildings in both developed and developing countries (Zhang et al., 2015). Most of these technologies and projects mainly focus on the technical and economic efficiency of nutrient recycling while only a few studies reported the hygienic aspect of urine. Actually, urine is not sterile and it contains culture-positive microbiological communities (Hilt et al., 2014). Several bacterial and viral pathogens were detected in the source-separated urine collected at the eThekwini Water and Sanitation municipality in South Africa (Bischel et al., 2015). Users of toilet and urine-derived fertilizer product (UDFP) may be exposed to pathogens-carrying urine and UDFPs. Moreover, consumers also may be exposed to food fertilized with UDFPs. All these possible exposure pathways can bring health risks to people who may be infected through vehicleborne transmission defined as one of the indirect transmission modes (US Department of Health and Human Services, 2012).

Quantitative Microbial Risk Assessment (QMRA) has been used to assess the microbial risk in handling of urine and eating vegetables fertilized by urine (Hoglund et al., 2002; Ahmed et al., 2017; Bischel et al., 2019; Oishi et al., 2020). Typical pathogens examined included *Campylobacter jejuni,* Enterotoxigenic *Escherichia coli (E. coli)*, *Shigella* spp., *Cryptosporidium parvum* and rotavirus, etc. All these studies reported health risks to users, workers and consumers when pathogens-containing urine was collected, transported and handled without any pretreatment. Storage for 2–6 months is usually recommended for effective inactivation of pathogens in urine, which is caused by high alkaline pH and high free ammonia due to urea hydrolysis (World Health Organization (WHO), 2006; Vinnerås et al., 2008). Moreover, pathogens in urine can also be effectively inactivated through alkaline treatment by ash and lime (Randall et al., 2016; Senecal et al., 2018). However, up to date, there still lacks a connection of urine treatment to its effect on pathogens inactivation, which might make researchers ignore the microbial risks of urine source separation especially developing new technologies for urine treatment. Particularly, the pandemic of Coronavirus Disease 2019 (https://www.who.int/emergencies/diseases/novel-coronavirus-2019) and the outbreak of more deadly disease caused by Monkeypox (https://www.who.int/emergencies/situations/monkeypox-oubreak-2022) strongly reminds the possible microbial risks in the recycling of urine nutrients in agriculture.

Therefore, this study aimed to review the inactivation of pathogens in urine treatment technologies. Pathogens in urine reported in publications were summarized. The inactivation of pathogens in various technologies and UDFPs were evaluated based on a comprehensive review of publications. Then, an overall assessment was carried out to build a connection of urine treatment to its effect on pathogens inactivation. Finally, an outlook was proposed for the microbial risk management in urine source separation and nutrient recovery.

2 Pathogens in urine

Only a few publications reported pathogens detected in urine collected from toilets while many reported findings in urine collected from patients in medical research. Bacteria and viruses detected in urine in literature have been briefly summarized in Supplementary Table S1. Therefore, urine is not sterile. Possible approaches for pathogens introducing to urine were discussed as following.

2.1 Pathogens excretion with urine

Human urine forms by filtration of blood plasma through glomerular filtration barrier into nephron in kidneys (Rose, 1966). The barrier commonly has nanoscale pores while sizes of bacteria are approximately of micron scale (Goswami and Pugazhenthi, 2020). For example, the length and the diameter of *E. coli* is approximately 2 μm and 0.5 μm, respectively (Sherbet and Lakshmi, 1973). Thus, urine often does not contain bacteria. However, urine will be contaminated by pathogenic bacteria when infection occurs in the urinary system of patients. Studies have reported several pathogenic bacteria in urine, which often cause some clinical urinary tract infection and inflammation, such as *E. coli* (Hinata et al., 2004), *Proteus mirabilis, Pseudomonas aeruginosa* (Qiao et al., 2013), a variety of gram-negative and gram-positive bacteria (Shigemura et al., 2005). Sun et al. (2020) identified more than 10^5/ml of bacterial particles in clinical urine samples through analysis by mass spectrometry combined with UF–5000i flow cytometry, and the pathogens were all single microbial species (Sun et al., 2020). Chen et al. (2018) successfully detected *E. coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* in urine samples, with the detection limit of 100 colony-forming unit (CFU)/mL, using multiplex recombinase polymerase chain reaction (Chen et al., 2018).

Infection of urinary system can also introduce viruses to urine similar as the introduction of bacteria. In addition, viruses usually range in size from 5 to 300 nm (Goldsmith and Miller, 2009) and most of them lies in the lower margin of the scale as summarized by (Goswami and Pugazhenthi, 2020). Thus, a part of viruses can pass through the glomerular filtration barrier in kidneys, thereby contaminating urine. For example, researchers reported that the diameter of SARS-CoV-2 ranged in 60–140 nm

(Zhu et al., 2020). The virus detection rate in the serum and urine samples collected from 74 COVID-19 patients were 2.8% (9/323) and 0.8% (2/247), with mean viral load of 1210 ± 1861 and 79 ± 30 copies/mL, respectively (Kim et al., 2020). Kim et al. (2020) did not report urinary system infection by SARS-CoV-2. This indicates that virus in serum passed through the barrier to urine. While the viral load in urine was low and the SARS-COV-2 could not be isolated from the virus-positive samples (Kim et al., 2020), the microbiological risk of viral infection still cannot be ruled out. In addition, researchers have found many types of viruses in urine, such as Zika virus (Wiwanitkit, 2016), SARS virus (Xu et al., 2005), dengue virus (Van den Bossche et al., 2015), BK virus (Konietzny et al., 2012), Rift Valley fever and yellow fever virus (Li M. et al., 2019), etc. Therefore, urine would inevitably contain pathogens due to pathogen permeation from serum and infection of urinary system when patients use toilets.

2.2 Fecal contamination

Besides urinal, urine diverting toilet, which is divided into two parts through a partition, is employed to collect urine separately from feces. This toilet is quite different from a conventional toilet, which may make users puzzled especially when there is insufficient instruction. Incorrect use or cleaning of the toilet may cause the feces to enter the urine, which is a common unavoidable phenomenon (Schonning et al., 2002). Moreover, insect vectors (e.g., mosquitoes and flies) can also be carriers of microbes from feces to urine. The detection of intestinal microbes such as *E. coli* in source-separated urine would be an evidence for the cross contamination by feces (An et al., 2020). A study found that the corresponding level of fecal contamination of urine samples in eco-villages was 38% and at public places (i.e., work places and schools) 58% of the urine samples were contaminated when the contamination was determined based on the content of fecal sterols (Schonning et al., 2002). Therefore, cross contamination of urine by feces can be confirmed to be another route to introduce pathogens to urine. Feces contain more than 100 different varieties of viruses, bacteria, and helminthes (Senecal et al., 2018). However, only a few kinds of bacteria have been detected in source-separated urine because of the lack of related studies.

Urine contamination by feces would enlarge the microbial risks of reusing urine in agriculture. As far as the SARS-COV-2 is concerned, it may replicate in human gastrointestinal tract, exist in fecal excrement for a certain period of time, and may have infectious pathogenicity (Senecal et al., 2018). Zhang et al. (2020) investigated the feces of 23 patients, 83% of those were positive and the average duration of virus shedding was 22 days. During this period, the average titer of virus in feces was 5,623 copies/mL, but the highest titer reached $10^{5.8}$ copies/mL at the peak (Zhang et al., 2020). Although the viral load in urine was quite low in the clinical study of sampling in aseptic environment (Kim et al., 2020), the viral load and its potential risk would increase a lot when urine was contaminated by feces in the field-scale projects of source separation. As such, cross contamination of feces is considered as a dominating risk of disease transmission when handling and reusing urine (Senecal et al., 2018). Therefore, due care should be taken in the design of new urine-diverting toilets, instructions of toilet use and training of urine collection users.

3 Pathogens in urine treatment and UDFPs

3.1 Direct use of urine after storage

Direct use of urine as liquid fertilizer is a commonly recommended simple approach for nutrient recycling from urine to agriculture. This has been successfully applied for the cultivation of microalgae (Adamsson, 2000; Golder et al., 2007), zooplankton (Adamsson, 2000) and various plants such as cucumbers (Heinonen-Tanski et al., 2007), lettuce, tomatoes (Adamsson, 2000; Mnkeni et al., 2008; Pradhan et al., 2009), okra (Akpan-Idiok et al., 2012), beet (Mnkeni et al., 2008; Pradhan et al., 2010a), pumpkin (Pradhan et al., 2010b), carrot (Mnkeni et al., 2008), maize (Guzha et al., 2005; Mnkeni et al., 2008; Antonini et al., 2012a), cabbage (Pradhan et al., 2007), water spinach (Yang et al., 2015), Lolium multiflorum Lam (Antonini et al., 2012a), wheat (Tidåker, 2003) and barley (Tidåker, 2003). However, the presence of pathogens may pose a potential risk for the users of fertilizer and the consumers of agriculture products. Particularly, the regrowth of some microorganisms such *E. coli* were even reported in fresh urine because of its appropriate pH and nutrients (Lahr et al., 2016; Ahmed et al., 2017).

To reduce the microbial risks of urine application, storage for 2–6 months is commonly recommended as a hygienic treatment method (Schonning, 2001; Jönsson et al., 2004; World Health Organization (WHO), 2006). Table 1 summarizes the inactivation of pathogens in urine after storage, including *Aeromonas hydrophila* (Höglund et al., 1998), Enterococci (Höglund et al., 2000), *Enterococcus faecalis* (Chandran et al., 2009; Nordin et al., 2013; Almeida et al., 2019), *E. coli* (Höglund et al., 1998; Höglund et al., 2000; Chandran et al., 2009; Wohlsager et al., 2010; Nordin et al., 2013; Ahmed et al., 2017; Zhou et al., 2017; Almeida et al., 2019; Oishi et al., 2020), fecal coliforms (Höglund et al., 2000; Wohlsager et al., 2010; Zhou et al., 2017; Almeida et al., 2019), fecal streptococci (Höglund, 2001; Wohlsager et al., 2010), human adenovirus (Decrey and Kohn, 2017), *Mycobacterium tuberculosis* (Vinnerås et al., 2011), *Mycobacterium* bovis (Vinnerås et al., 2011), *Pseudomonas aeruginosa* (Höglund, 2001), *Salmonella typhimurium* (Höglund et al., 1998; Vinnerås et al., 2008; Chandran et al., 2009; Wohlsager et al., 2010), *Salmonella senftenberg* (Höglund et al., 1998), sulphite reducing *Clostridia*

TABLE 1 A summary on the reduction of pathogens in storage of urine.

Urine	Temperature°C	Pathogens	Reduction		Days	References
			Description	Unit		
Diluted and undiluted	15–35	*Escherichia coli*	5.5 log10	CFU/mL	3–50	Ahmed et al. (2017)
		coliphage MS2	5.2 log10	PFU/mL	2–50	
Diluted	5–20	*Rhesus* rotavirus	90%	[a]	32–240	Höglund et al. (2002)
		Salmonella typhimurium			71–1466	
Diluted and undiluted	4–20	*Aeromonas hydrophila*	6 log10	CFU/mL	1–20	Höglund et al. (1998)
		Pseudomonas aeruginosa				
		Salmonella senftenberg				
		Salmonella typhimurium				
		Escherichia coli	1 log10	CFU/mL	1–14	
		Ascaris suum	15%–20%		0–21	
		faecal streptococci	1 log10	CFU/mL	6–19	
Diluted	4	*Cryptosporidium parvum* oocysts	90%		29	Höglund and Stenström, (1999)
Undiluted	20	*Ascaris suum* egg	1 log10-3 log10	Eggs/mL	24–90	Senecal et al. (2018)
		Salmonella typhimurium	1 log10-6 log10	CFU/mL	9–53	
		Enterococcus faecalis			26–155	
		Bacteriophages MS2		PFU/mL	1–6	
		Bacteriophages ΦX 174			29–175	
Diluted and undiluted	15–30	*Escherichia coli*	6.7 log10	CFU/mL	0–63	Chandran et al. (2009)
		Salmonella typhimurium	6.8 log10		7–49	
		Enterococcus faecalis	8.4 log10		0–69	
		coliphage MS2	8.6 log10	PFU/mL	7–42	
Diluted	21–24	*Ascaris suum* eggs	3 log10	Eggs/mL	1–10	Nordin et al. (2013)
		Enterococcus spp.	90%	[a]	7–10	
		Enterococcus faecalis			2	
		Salmonella typhimurium			0.1	
		Escherichia coli			0.1	
		enterobacteria phage MS2			8–16	
		coliphage ΦX 174			37–43	
		Salmonella typhimurium phage 28B			55–64	
Diluted and undiluted	4–30	total coliforms	3–5 log10	CFU/mL	0–90	Almeida et al. (2019)
		Escherichia coli	3–4 log 10			
		Enterobacteriaceae	5–6 log10			
		Enterococcus	4 log10			
		total staphylococci	6 log10			
Diluted and undiluted	27	total coliforms	5.4 log10	CFU/100 ml	0–28	Wohlsager et al. (2010)
		Escherichia coli	4 log10		0–28	
		Faecal *streptococci*	6.1 log10		0–56	
Diluted	60–70	fecal coliforms	3 log10	CFU/mL	0–1	Zhou et al. (2017)
		Escherichia coli	4.3 log10		0–3	
Diluted and undiluted	4–34	*Salmonella typhimurium*	90%	[a]	0–33	Vinnerås et al. (2008)
		Enterococcus faecalis			1–47	
		bacteriophages MS2			1–240	
		bacteriophages ΦX 174			5–150	
		bacteriophages 128			1–169	
Hydrolyzed	20	BK human polyomavirus	90%	[a]	0–9	Goetsch et al. (2018)
		bacteriophages T3			24–46	

(Continued on following page)

TABLE 1 (*Continued*) A summary on the reduction of pathogens in storage of urine.

Urine	Temperature°C	Pathogens	Reduction		Days	References
			Description	Unit		
		bacteriophages Qβ			0.3	
		bacteriophages MS2			0.4	
Diluted and undiluted	4–34	*Ascaris suum* eggs	99%	[a]	3–840	Nordin et al. (2009)
Diluted	4–22	*Mycobacterium tuberculosis*	90%	[a]	2–10	Vinnerås et al. (2011)

[a]The reference doesn't provide any information on the unit of pathogens tests.

(Höglund et al., 2000; Almeida et al., 2019), BK Human Polyomavirus (Goetsch et al., 2018), *Rhesus rotavirus* (Höglund et al., 2002), *Ascaris suum*, *Ascaris suum* egg (Nordin et al., 2009), and *Cryptosporidium parvum* oocysts (Höglund and Stenström, 1999). Furthermore, inactivation experiments sometimes used bacteriophage as virus surrogate, such as bacteriophage T3 (Goetsch et al., 2018), coliphage ΦX174 (Vinnerås et al., 2008; Nordin et al., 2013; Decrey and Kohn, 2017), coliphage MS2 (Chandran et al., 2009; Decrey et al., 2015; Ahmed et al., 2017; Decrey and Kohn, 2017), coliphage T4 (Decrey and Kohn, 2017), *Enterobacteria* phage MS2 (Vinnerås et al., 2008; Nordin et al., 2013), and *Salmonella typhimurium* phage 28B (Höglund et al., 2002; Vinnerås et al., 2008). Most of these pathogens were reduced to an acceptable limit after 6 months.

Several factors may respond for the pathogens inactivation, of which free ammonia (NH_3) induced by the hydrolysis of urea in the storage is considered as the dominating reason (Vinnerås et al., 2008). NH_3 is one of the most important basic species in stored urine inducing the genome cleavage *via* alkaline transesterification, which results in the inactivation of the single-stranded (ss)RNA viruses (Decrey et al., 2015). Based on the same mechanism, other bases (carbonate, phosphate, and hydroxide) should also be taken into account for the viruses inactivation (Decrey et al., 2015). Furthermore, high temperature and high pH can accelerate the inactivation because these two factors also determine the concentrations of NH_3 and other bases (Höglund et al., 1998; Höglund and Stenström, 1999; Hoglund et al., 2002; Vinnerås et al., 2008; Ahmed et al., 2017). For example, the inactivation rate constant *K* of *E. coli* increased from 0.20/d to 3.04/d at increasing temperature from 15°C to 35°C in storage of undiluted urine (Ahmed et al., 2017). 20°C or higher temperature was recommended for the current recommended storage time of 6 months (Vinnerås et al., 2008). Moreover, urine dilution brought by water flushing or pretreatment before fertilization (Jönsson et al., 2004) can decrease the concentration of NH_3, thereby slowing the pathogens inactivation and extending the storage time for hygienic treatment (Vinnerås et al., 2008).

Notably, the types of pathogens of which the inactivation was experimentally studied (Table 1) are far less than that detected in urine (Supplementary Table S1). The inactivation of pathogens will be surely determined by its type. For example, the mean time for 1 log10 reduction (t_{90}) of *S. typhimuriuma*, *Enterococcus Faecalis*, MS2, and ΦX174 was <0.1 d, <1.1 d, <1.6 d, and <5.7 d, respectively, during the storage of urine at 34°C (Vinnerås et al., 2008). Some pathogens were still in high concentrations and even enriched after several months of storage. The concentration of *Bacillus* spores did not decrease during the urine storage because some microorganisms in the sporulated form have a higher resistance (Almeida et al., 2019). Moreover, the growth of streptococci was even found in urine collecting pipes and tank (Höglund et al., 1998). In addition, heterogeneity among viruses in their susceptibility toward NH_3 is driven by the genome type. It is more difficult to inactivate the double-stranded (ds)RNA reovirus and *Rhesus rotavirus* as well as the dsDNA *Salmonella* phage 28B than ssRNA viruses (Decrey et al., 2015). Therefore, it is essential to carry out a comprehensive evaluation of the inactivation of possible pathogens particularly in areas with typical infectious diseases when urine was directly used as liquid fertilizer after storage.

In addition, several pretreatment methods were developed to accelerate the urea hydrolysis, which may accelerate the pathogens inactivation. These methods included dosage of urease (Kabdasli et al., 2006; Engelhardt et al., 2020), fecal incubation (Hotta and Funamizu, 2008), incubation of stale urine (Liu et al., 2008), increasing temperature (Liu et al., 2008) and microbial electrochemical current (Chen et al., 2017). However, incubation of fecal matter can simultaneously bring the contamination of urine by pathogens whether more pathogenic types or higher concentrations. As such, the pretreatment using fecal incubation should be re-considered taking the microbial risks into account.

3.2 Urine stabilization

Urea hydrolysis driven by the catalysis of urease occurred quickly in urine because many bacteria contaminated by feces or

nature can produce urease (Udert et al., 2003a). More than half of urea hydrolyzed in the urine collecting pipes and storage tank (Udert et al., 2003b; 2003c). Although urea hydrolysis promotes the pathogens inactivation, it also brings the loss of nitrogen due to ammonia volatilization and the loss of phosphorus due to phosphate precipitation at alkaline pH. To inhibit urea hydrolysis, stabilization has been developed as a pretreatment before urine reuse or dewatering (Randall et al., 2016).

3.2.1 Acidification/alkalinization

Acidification (pH < 5 (Hellstrom et al., 1999; Saetta et al., 2020; Moharramzadeh et al., 2022)) or alkalinization (11 < pH < 13 (Randall et al., 2016)) can be used to inhibit the hydrolysis of urea due to the inactivation of urease-producing bacteria or urease. The inactivation of pathogens after urine acidification has been rarely reported maybe because the disinfection is not significant. By comparison, alkalinization of urine is considered as a promising method to inactivating pathogens as well as stabilizing urine (Randall et al., 2016). Lime is a common alkaline agent used for urine stabilization (Randall et al., 2016), sometimes combining with biochar (Simha et al., 2020a) and wood ash (Dutta and Vinnerås, 2016; Simha et al., 2020b). Pathogens inactivation in urine using lime has not been experimentally examined. However, lime is a well-known disinfection method for water and sewerage sludge because high pH was highly effective in the inactivation of pathogens (Bujoczek et al., 2002; Keller et al., 2004; Hansen et al., 2007). Moreover, bacteria *Enterococcus faecalis* and *Salmonella* spp. and bacteriophages MS2 and ΦX 174 were reduced to below the detection limit when urine was alkalized using wood ash after 4 d at 20°C (Senecal et al., 2018) while the mean time for t_{90} of *Enterococcus Faecalis*, MS2, and ΦX174 was 2.3, 15, and 12 d, respectively, during urine storage at 24°C (Vinnerås et al., 2008). Therefore, alkalinization would be much quicker and more effective than urine storage.

3.2.2 Chemical oxidation

Chemical oxidation using hydrogen peroxide or heat-activated peroxydisulfate could effectively inhibit urea hydrolysis due to the irreversible destruction of urease (Zhang et al., 2013; Lv et al., 2020). The two oxidants are normally used in disinfection of water (Jin et al., 2020; Xiao et al., 2020), providing a basis for pathogens inactivation of urine. However, chemical oxidation also brought the loss of nitrogen because of the oxidation of ammonium to nitrogen gas (Lv et al., 2020). This might limit the application of chemical oxidation for urine stabilization.

3.2.3 Biological nitrification

Urine after urea hydrolysis can also be stabilized through biological nitrification. Ammonium in hydrolyzed urine can be oxidized to nitrite or nitrate while the pH decreases to a weak acidic pH (i.e., 5–7) (Udert et al., 2003d; Feng et al., 2008; Jiang et al., 2011). This would reduce the volatilization of ammonia and the precipitation of phosphate salts. Furthermore, this produces a stabilized liquid fertilizer (Feng et al., 2008) or solid fertilizer when combining with distillation (Udert and Wächter, 2012; Fumasoli et al., 2016). However, the impact of nitrification on pathogens inactivation is not an interest even since nitrification was proposed for biological nitrogen removal from wastewater (Gujer, 2010). This means that biological nitrification may not be an effective technology on pathogens inactivation in urine. Notably, a recent study achieved the nitritation of urine in an acidic nitrifying bioreactor (Li et al., 2020). This may provide an opportunity to inactivate pathogens in urine because the product free nitrous acid is biocidal to a broad range of microorganisms (Jiang and Yuan, 2014) despite the target of nitritation was to obtain nitrite rather than recover nutrients from urine.

3.3 Dewatering urine to obtain concentrated fertilizer products

Despite being rich in nutrients, urine still has high content of water (approximately 97%) when urine is collected in a dry toilet. Urine will be further highly diluted when a water flushing toilet is used. Several dewatering technologies have been developed to concentrate urine to yield concentrated fertilizer products as well as clean water.

3.3.1 Membrane filtration

Urine can be dewatered to produce concentrated liquid fertilizer through membrane filtration including nanofiltration (Ray et al., 2020a; Courtney and Randall, 2022), reverse osmosis (Ek et al., 2006; Ray et al., 2020a; Courtney and Randall, 2022), forward osmosis (Zhang et al., 2014; Engelhardt et al., 2020; Jiang et al., 2022; Pocock et al., 2022), membrane distillation (Zhao et al., 2013; Volpin et al., 2019a; Khumalo et al., 2019) and electrodialysis (Brewster et al., 2017; Wang et al., 2017; De Paepe et al., 2018). Nutrients are rejected in the concentrate while water passes through the membrane filter to the other side in most of the filtration processes except the electrodialysis which concentrates nutrients in the permeate side.

Membrane filtration is a common technology to remove pathogens from water. The separation of bacteria and viruses from wastewater using polymeric and ceramic membrane has been summarized (Goswami and Pugazhenthi, 2020). The rejection of pathogens by filter mainly depends on the filter pore size due to the size exclusion mechanisms. Moreover, virus adsorption, electrostatic interaction and hydrophobic interaction also respond for the removal of pathogens from water (Goswami and Pugazhenthi, 2020). A nanofilter with pore size of 20 nm (Viresolve NFP filter, Millipore, United States) was employed to remove viruses from urine aiming to obtain virus free urokinase solution. The log reduction factors for porcine parvovirus (18–26 nm), human hepatitis A virus (25–30 nm), murine

encephalomyocarditis virus (28–30 nm), bovine viral diarrhoea virus (40–60 nm), and bovine herpes virus (120–300 nm) were ≥4.86, ≥4.60, ≥6.87, ≥4.60, and ≥5.44, respectively (Kim et al., 2004). The rejection of pathogens by filter also means that pathogens would be concentrated in the liquid urine fertilizer. A recent study reported that concentration of urine can also lead to the inactivation of *E. coli* due to the high osmotic pressure under high salt contents (Oishi et al., 2020). 1-day storage was necessary for the safe reuse of 5-fold concentrated hydrolyzed urine whereas 91-h storage was required for non-concentrated urine when the initial numbers of *E. coli* were the same in these two urine solutions. However, the inactivation of pathogens might be inhibited due to the low concentration of free ammonia at low pH when acidification pretreatment was used to reduce the loss of ammonia to the water permeate (Tun et al., 2016; Xu et al., 2019a; Ray et al., 2020a).

Electrodialysis may pose an advantage than other membrane filtrations for dewatering urine. Nutrients were concentrated in the permeate side as the ionic species passed through selective ion-exchange membranes from the urine to the permeate side driven by an electric field (Pronk et al., 2006; De Paepe et al., 2018). Thus, pathogens would be retained in urine while yielding a pathogen free fertilizer product. However, this has not been examined by experiments.

Forward osmosis and membrane distillation were also employed to separate ammonia and urea from urine, producing ammonia- or urea-rich solution in the permeate side (Xu et al., 2019a; Ray et al., 2019; Ray et al., 2020b; Han et al., 2022). The N-rich solution would have low pathogen concentrations due to the rejection by the filter. Furthermore, a P-rich solid product could be obtained in a pretreatment using struvite precipitation before the filtration to avoid fouling on the membrane (Volpin et al., 2018; Volpin et al., 2019b). The combined processes successfully would achieve the recovery of N and P from urine. However, special attention should be paid to microbial risks of the struvite product formed in the urine with concentrated pathogens.

3.3.2 Evaporation/distillation

Urine can be passively evaporated at ambient temperature to produce concentrated fertilizer solution or solid fertilizer products (Bethune et al., 2014). The evaporation was further accelerated by solar irradiation (Antonini et al., 2012b; Bethune et al., 2015) or heating at 25–65°C (Dutta and Vinnerås, 2016; Senecal and Vinnerås, 2017; Simha et al., 2018; Simha et al., 2020a). The purpose of urine distillation at boiling temperature is mainly to obtain solid fertilizer products (Udert and Wächter, 2012; Fumasoli et al., 2016; Jiang et al., 2017). The boiling temperature even increased to 130.1°C when the salts in urine was highly concentrated following the distillation (Udert and Wächter, 2012). However, loss of the total ammonia was observed due to the volatilization of free ammonia during the evaporation of water (Bethune et al., 2014,2016). Therefore, stabilization pretreatment such as acidification (Jiang et al., 2017), alkalinization (Dutta and Vinnerås, 2016; Senecal and Vinnerås, 2017; Simha et al., 2018; Simha et al., 2020a) and nitrification (Udert and Wächter, 2012; Fumasoli et al., 2016) was usually employed prior to the evaporation or distillation process.

Several factors involved in the evaporation/distillation of urine can inactivate pathogens. High temperature is highly effective to inactivate pathogens as reported in the fields of waste composting or water disinfection (Chu et al., 2019; Hu et al., 2020). Moreover, Bethune et al. (2014) suggested that the urine-derived solid product was sterile because high salinity in the concentrated product might respond for the inactivation of microorganisms. In addition, solar thermal evaporation may further enhance the pathogen inactivation because sunlight radiation is considered as a unconventional disinfection technology to obtain safe drinking water (Chu et al., 2019). The inactivation of pathogens would be further enhanced when alkaline pretreatment was employed to inhibit the urea hydrolysis before evaporation/distillation. In an alkaline dehydration of urine using ash at 42°C, five fecal pathogens including *Ascaris suum*, *Enterococcus faecalis*, bacteriophages MS2 and ΦX 174, and *Salmonella* spp. were inactivated to below the detection limit within 10 d (Senecal et al., 2018), which met the World Health Organization (WHO) guidelines for the reuse of excreta (World Health Organization (WHO), 2006). Although acidification or biological nitrification may be not effective on the pathogen inactivation, the following evaporation/distillation can play a dominating role (Bischel et al., 2015).

The evaporation/distillation of urine can also produce clean water for reuse. Distillation at boiling temperature would also promise a sterile water product because boiling temperature is very efficient on disinfection (Chu et al., 2019). However, reclaimed water collected after evaporation at ambient temperature or high temperature around 35–65°C may still pose microbial risks. Pathogens in the concentrated fertilizer solution or the solid product may be inactivated by the synergistic effects of high salinity, pH and temperature. But single effect of temperature on the water disinfection may not act as effectively as on the solid product disinfection. Thus, the reuse of the reclaimed water after the evaporation/distillation of urine should be further addressed.

3.3.3 Freezing-thawing

Freeze crystallization can recover water in the form of ice from urine as well as concentrating the fertilizer solution. Low temperature ranges of −18 to −4°C were examined for freezing urine (Lind et al., 2001; Gulyas et al., 2004; Schmidt and Alleman, 2005; Ganrot et al., 2007a; Yu et al., 2007; Ganrot et al., 2008; Moharramzadeh et al., 2022). A thermodynamic model was further developed providing a theoretical understanding on obtaining solid salts and water from stored urine (Randall and Nathoo, 2018). After thawing, the reclaimed water and the concentrated fertilizer product can be obtained.

The influence of freezing and thawing on pathogens seems to be unclear. Freezing creates damage to cell structure of microorganisms (Sanin et al., 1994). It was found that the reduction of pathogenic bacteria (*Salmonella*), virus (Poliovirus) and parasites (*Ascaris suum* and *Cryptosporidium parvum*) was effectively achieved by freezing-thawing coupled with anaerobic digestion of sludge in a wastewater treatment plant, meeting the pathogen reduction requirements for sludge land disposal by US regulations (Sanin et al., 1994). Furthermore, freezing is widely used in food storage because of its negative effect on microbial community. For examples, storage for more than 3 days at −20°C can effectively inactivate *T. gondii* in contaminated meat (Alizadeh et al., 2018). However, freeze-drying is also considered as a common cryopreservation method to preserve pathogens because the ultra-low temperature can fix the cells in powder and nearly completely inhibit the cell metabolism (Perry, 1998). For example, the freeze-drying was sustainable for the long-term preservation of marine pathogen *V. anguillarum* (Yu et al., 2019). Furthermore, the stability and retention of infectivity of viruses was confirmed after frozen storage of foods, indicating negative effect on killing viruses (Sánchez and Bosch, 2016). In addition, it was reported that sublethally injured pathogens (*E. coli*) caused by freezing during food storage can recover after thawing, presenting a potential threat on food safety (Zhang R. et al., 2021). Therefore, the related publications can't show a clear understanding whether freeze-drying is bad or good to effectively deactivate pathogens in urine.

3.4 Extraction of nutrients from urine

3.4.1 Stripping-absorption of ammonia to obtain liquid N-products

Ammonia separated from urine by air (Behrendt et al., 2002; Basakcilardan-Kabakci et al., 2007; Antonini et al., 2011; Morales et al., 2013; Liu et al., 2015; Xu et al., 2017; Jagtap and Boyer, 2018; Jagtap and Boyer, 2020) or membrane stripping (Tarpeh et al., 2018a; Christiaens et al., 2019; Pradhan et al., 2019) can be absorbed in water or sulfate acid solution to yield liquid N-products such as ammonia water or ammonium sulfate solution. However, an autofluorescent *E. coli* spiked in the urine was also observed in the absorbent (Christiaens et al., 2019), which should be caused by the transfer of microorganisms from urine to the absorbent *via* aerosols induced by air stripping (Benami et al., 2016). This indicates potential microbial risks of using the ammonia absorbent products in agriculture. Alkalinization and heating is usually used to improve ammonia stripping efficiency from urine. pH >12 maintained by lime increased the removal efficiency by 8% for the stripping of pure urine at 30°C (Pradhan et al., 2017). Furthermore, high temperature ranging in 40°C–65°C could significantly increase the stripping efficiency because of the formation of more free ammonia (Pradhan et al., 2017; Wei et al., 2018; Tao et al., 2019; Tian et al., 2019). High pH and temperature would meanwhile benefit the inactivation of pathogens in urine influent as well as reducing the pathogens in absorbent.

Membrane stripping provide another approach to reduce the transfer of pathogens to absorbent. Free ammonia in the urine side pass through a gas permeate membrane (GPM) to the absorbent side. Pore size of the GPM commonly ranges in 0.1–0.59 μm (Tarpeh et al., 2018a; Xu et al., 2019a; Christiaens et al., 2019). As such, most pathogenic bacteria and some viruses would be rejected by the membrane, producing N-products with less pathogens in the permeate side. A study by Tarpeh et al. (2018a) confirmed that *E. coli* was not observed in the absorbent of a membrane stripping process when its concentration was $10^{8.00-8.78}$ events/mL in the urine feed. However, it was also found that very small microorganisms reported in urine (<0.1 μm) could pass through the membrane with an average measured pore size of 0.274 μm (Dong et al., 2011). Thus, stripping *via* hydrophobic GPM does not lead to a pathogens-free absorbent. Similar as that in the air stripping process, high pH and temperature intending for high ammonia transfer efficiency in membrane stripping (Xu et al., 2019a) would probably inactivate pathogens in the feed side and reduce the transfer of pathogens to the permeate side.

Concentrating ammonia using cation exchange membrane (CEM) in electrodialysis can increase the concentration of free ammonia before stripping (Christiaens et al., 2017; Tarpeh et al., 2018a; Christiaens et al., 2019). Tarpeh et al. (2018a) developed a novel process combining electrodialysis and membrane stripping to yield an *E. coli*-free fertilizer product from urine for potential safe reuse. Pore size of CEM ranges from 1.5 to 200 nm (Kononenko et al., 2017), which would benefit rejecting the transfer of pathogens with small sizes such as nanoscale viruses.

3.4.2 Adsorption of nutrients to obtain solid fertilizer products

Urea, ammonium, phosphate and potassium in urine can be adsorbed by various adsorbents such as activated carbon (Ganrot et al., 2007a), biochar (Bai et al., 2018; Xu et al., 2018; Xu et al., 2019b; Zhang X. et al., 2021), gastropod shell (Saliu et al., 2020), ion exchange resin (O'Neal and Boyer, 2013; O'Neal and Boyer, 2015; Tarpeh et al., 2017; Tarpeh et al., 2018b), layered double hydroxides (LDH) (Dox et al., 2019a; Dox et al., 2019b; Dox et al., 2022), magnetic Fe_3O_4@ZrO_2 nanoparticles (Guan et al., 2020a), metal organic frameworks (MOF) (Lin et al., 2015; Guan et al., 2020b), natural loess (Jiang et al., 2016), porous organic polymer (Zhang et al., 2020), wollastonite (Lind et al., 2000), zeolite (Ban and Dave, 2004; Beler-Baykal et al., 2004; Ganrot et al., 2007a; Leung et al., 2007; Ganrot et al., 2008; Baykal et al., 2009; Beler-Baykal et al., 2011; Huang et al., 2014; Xu S. et al., 2015; Mitrogiannis et al., 2018; Makgabutlane et al., 2020; Regmi and Boyer, 2020). Some of these adsorbents i.e., biochar, gastropod shell, natural loess, and zeolite after nutrient

adsorption can be directly used as fertilizer in agriculture (Ganrot et al., 2008; Beler-Baykal et al., 2011; Xu S. et al., 2015; Jiang et al., 2016; Bai et al., 2018; Xu et al., 2018; Saliu et al., 2020; Zhang X. et al., 2021). Other adsorbents should be desorbed in solutions containing NaOH (Beler-Baykal et al., 2004; O'Neal and Boyer, 2015; Mitrogiannis et al., 2018; Guan et al., 2020a; Guan et al., 2020b), $NaHCO_3$ (Mitrogiannis et al., 2018; Dox et al., 2019a; Dox et al., 2019b), NaCl (Lin et al., 2015; O'Neal and Boyer, 2015; Mitrogiannis et al., 2018; Zhang et al., 2020), HCl (Mitrogiannis et al., 2018; Guan et al., 2020b), or H_2SO_4 (Tarpeh et al., 2017; Tarpeh et al., 2018b) to obtain phosphate-rich solution, after which a chemical precipitation process such as struvite precipitation was usually conducted to yield solid phosphate fertilizer easy to be collected and applied for agriculture. However, to our best knowledge, the behavior of urine pathogens during the nutrient adsorption has not been reported.

Normally, some adsorbents such as zeolites and biochar can uptake aquatic microorganisms, forming biofilm on the surface (Perez-Mercado et al., 2019; Wang et al., 2020). The uptake of microorganisms is mainly driven by weak electrostatic or binding forces to the carrier surface and the intra-grain and intra-crystallite pores (Lameiras et al., 2008). Experiments confirmed the uptake of fecal coliforms and *E. coli* from wastewater by clinoptilolite, a naturally occurring zeolite, with an active uptake of 3989 CFU/g and 2732 CFU/g, respectively (Ferronato et al., 2015). Furthermore, a correlation model based on experiments also showed that biochar filters could significantly remove bacteria and viruses with the reduction rate of ≥2 log10 and ≥1 log10, respectively, because these microbes were enriched on biochar due to adsorption (Perez-Mercado et al., 2019). This indicates that the microbial risks would even be magnified especially when the nutrient-enriched adsorbents are directly used as fertilizer in agriculture.

Various metal ions have been used as biocidal agents such as Ag, Zn, Cu, Ni, Co, Ti and Bi (Wyszogrodzka et al., 2016). Zeolite exchanged with Cu and Zn can be used as antimicrobial material for efficient disinfection of contaminated water with bacteria because it was effective to reduce the concentrations of *E. coli* and *Staphylococcus aureus* (Yao et al., 2019). This may provide a potential approach to reduce the microbial risks of nutrient-adsorbed solid fertilizer but pose a potential risk of heavy metals. Post treatment in an alkaline desorption solution may further inactivate pathogens adsorbed on adsorbents because alkaline treatment is effective in the disinfection of urine pathogens (Senecal et al., 2018). However, it is unclear that whether desorption of nutrients from adsorbents in acid solution could inactivate pathogens.

3.4.3 Chemical precipitation to obtain solid products

Chemical precipitation is a commonly reported approach to recover nutrients from urine by the formation of non-soluble phosphate salts. Struvite precipitation is the most concerned phosphorus recovery technology which can also simultaneously recover part of ammonium from urine (Krähenbühl et al., 2016; Zamora et al., 2017; Wei et al., 2018; Zheng et al., 2018; Aguado et al., 2019; Li P. et al., 2019; Pinatha et al., 2020; Oztekin et al., 2022). The precipitation of struvite-K, an analogue of struvite, can simultaneously recover phosphate and potassium from ammonium-depleted urine (Xu et al., 2011; Xu et al., 2012; Xu K. et al., 2015; Xu et al., 2017; Zhang et al., 2017; Zhang et al., 2018; Huang et al., 2019). Furthermore, phosphate can also be recovered by the precipitation of calcium phosphate (Pradhan et al., 2017). Finally, filtration and drying following the precipitation process is essential to obtain solid phosphate products. Struvite and struvite-K can be directly used as fertilizer in agriculture because they are both slow-release fertilizer while calcium phosphate is considered as a raw material for phosphate fertilizer industry (Ganrot et al., 2007b; Liu et al., 2020). There are standards for microbial risk management in industrial fertilizer (Ministry of Industry and Information Technology of the People's Republic of China, 2019). Therefore, this review mainly addresses the potential microbial risk of the recovered phosphate products which can be directly used in agriculture.

Decrey et al. (2011) found that the struvite precipitation process did not considerably inactivate the pathogen indicators phage ΦX174 and *Ascaris suum* eggs in the urine liquid fraction. The pH value during the struvite precipitation in urine was approximately 9 (Etter et al., 2011), which is similar as that in the storage process. This indicates that free ammonia formed at such alkaline pH can inactivate pathogens like that in the storage process. However, the reaction time for struvite formation was only 10 min in their study (Decrey et al., 2011). Some other studies used longer precipitation time ranging from 15 to 125 min (Ronteltap et al., 2007; Ronteltap et al., 2010; Liu et al., 2013), which is still much less that the storage time of 2–6 months (Jönsson et al., 2004; Vinnerås et al., 2008). As such, the pathogen inactivation can be neglected within such a short precipitation time. At a longer precipitation time of 24 h, the number of coliforms was reduced by 56% during the struvite precipitation at pH 9 in anaerobically digested chicken slurry (Muhmood et al., 2018). Furthermore, they also reported a greater reduction of coliforms (73%) at pH 10. The viability of spiked *E. coli* cells also significantly decreased during the struvite precipitation at pH of 11 in source-diverted blackwater (Yee et al., 2019). Non-etheless, optimum pH of the struvite precipitation was approximately 9 and higher pH decreased the struvite purity (Harada et al., 2006). Thus, high pH for effective pathogen inactivation may be suitable for precipitation of calcium phosphate rather than the struvite precipitation. Complete inactivation of *E. coli*, *Salmonella* enterica serovar typhymurium, and Porcine circovirus type 2 was achieved when removing phosphate from swine slurry at pH 10 using $Ca(OH)_2$ (Viancelli et al., 2015). The coliforms was also completely inactivated after 6 h of treatment using wood ash

to remove phosphate from urban wastewater and landfill leachate at pH of 10.1–12.7 (Ivanković et al., 2014). In addition, the optimal pH in ranges of 10–11 for the precipitation of struvite-K (Xu et al., 2011; Xu et al., 2012; Xu K. et al., 2015) may also improve the pathogens inactivation in the liquid fraction despite this has not been reported.

It is essential to evaluate the pathogens in the solid nutrient product obtained from urine especially when the product is directly used as fertilizer. Pathogen indicators phage ΦX174 and *Ascaris suum* eggs were both detected in the struvite product filtered by nylon fabric while no considerable inactivation was observed in the precipitation process in urine (Decrey et al., 2011). There was no significant difference on concentration of phage ΦX174 in both the solid and the liquid fraction. However, the *Ascaris* eggs even accumulated by 100 fold within the solid product because the filter with irregular pore diameters of 18–240 μm rejected the *Ascaris* eggs having a size of 35–50 × 45–70 μm in the solid fractions. Bischel et al. (2016) also reported the accumulation of heterotrophs and total bacteria in the struvite product obtained by the filtration with nylon or cloth filters from urine. Non-etheless, these two publications cannot clearly display an actual behavior of the pathogens in the solid fraction of struvite precipitation because of the effect of filtration on the pathogens accumulation. Electrostatic attraction and repulsion may play an important role for behavior of pathogens in solid struvite. Struvite crystals are negatively charged with zeta-potential of −17.5 to −27.6 mV at pH from 8.5 to 10.5 (Le Corre et al., 2007). Yee et al. (2019) reported that the electrostatic attraction between *C. perfringens* with positive zeta potential of 0–3 mV and struvite particles increased the cell aggregation in the final solid product. However, there was an electrostatic repulsion between struvite and phage ΦX174 with isoelectric point of 6.6 (Michen and Graule, 2010) carrying negative surface charge at pH around 9 (Muhmood et al., 2018). Moreover, isoelectric points of most viruses ranged from 1.9 to 8.4 (Michen and Graule, 2010). Thus, these viruses would present negative surface charge in the struvite precipitation system of urine, indicating electrostatic repulsion between struvite and viruses.

Factors affecting the surface charge of struvite may change the adsorption of pathogens onto struvite particles. High concentration of residual Mg^{2+} lowered the negative charge of struvite which could lead to more adsorption of pathogens with low isoelectric point (Decrey et al., 2011). Coagulants and flocculants may also result in more adsorption of pathogens when they were used to promote the agglomeration of struvite crystals (Le Corre et al., 2007). The adsorption of pathogens would probably be enhanced during the precipitation of struvite on metal-modified biochar (Xu et al., 2018; Liu et al., 2020) or zeolite (Huang et al., 2014; Mitrogiannis et al., 2018) with positive surface charge. In addition, the tendency of bacteria growth on these carrier (Perez-Mercado et al., 2019; Wang et al., 2020) would further lead to accumulation of more pathogens in the struvite product.

Although there lacks understanding on the adsorption of pathogens on struvite and the following filtration rejects some pathogens with large size in the struvite filter cake, pathogens in the solid products can still be effectively inactivated in the following drying process (Bischel et al., 2016). Temperature and humidity were important factors determining the efficiency of pathogens inactivation. No significant inactivation of *Ascaris suum* eggs in struvite obtained from urine were observed at 5 and 20°C while the eggs was inactivated by more than 99% at 35–36°C after drying for 3 days (Decrey et al., 2011). Furthermore, inactivation of *Ascaris suum* eggs was only 1.2 log10 after 3 days drying at temperature of 35°C and relative humidity of 85% while it reached more than 2 log10 after 1 day of drying at temperature of 36°C and relative humidity of 36%. Thus, inactivation of *Ascaris suum* eggs in struvite drying was enhanced at low relative humidity (Decrey et al., 2011). Moreover, the reduction of phage ΦX174 in struvite was 0.03, 0.07 and 0.09 log10/day in the drying process at 5°C/85% (temperature/relative humidity), 20°C/93% and 5°C/35%, respectively. As such, its inactivation was enhanced at high temperatures and low relative humidity. Experimental results further showed that the inactivation of heterotrophic bacteria, total bacteria, *Enterococcus* spp. and *Salmonella typhimurium* accumulated in struvite from urine was also improved with increasing temperature for drying at a constant relative humidity (Bischel et al., 2016). The reduction of heterotrophs even reached 3 log10 at optimized drying conditions within 100 h. Therefore, drying, also as an essential process to obtain final solid struvite product, is recommended as an effective method for the disinfection of the final product. Experimental results confirmed that the concentrations of total coliforms and *E. coli* were under the detection limits when struvite formed in anaerobically digested chicken slurry was dried at 40°C without control of humidity for 48 h after a filtration using 0.45 μm membrane (Muhmood et al., 2018; Muhmood et al., 2019). Drying at higher temperature than the ambient temperature already examined in experiments will reasonably improve the pathogens inactivation of struvite product. However, this may also result in the loss of crystal water and ammonia in the struvite crystals due to its decomposition (Bhuiyan et al., 2008).

Drying struvite by sunlight is also an option especially for small-scale reactor. However, Decrey et al. (2011) found that no significant inactivation of phage ΦX174 or *Ascaris suum* eggs was observed when struvite was exposed to sunlight within 5 h at 31°C with relative humidity of <35%. The short exposure time may be one of the dominating reasons for ineffective inactivation. Exposure to sunlight will increase the temperature of struvite and accelerate the loss of struvite moisture, thereby theoretically introducing the disinfection of struvite. Moreover, sunlight radiation in ultraviolet range is known to be effective in pathogens inactivation (Chu et al., 2019). Therefore, struvite disinfection is expected to be realized to some extent for long time of exposure in sunlight.

TABLE 2 An overall assessment of pathogens inactivation in urine treatment technologies and the related UDFPs.

Technology				Nutrient recovered	UDFP	Inactivation[a]
Urine storage				Complete	Liquid urine	++
Urine stabilization						
	Acidification			Complete	Liquid urine	N.I.
	Alkalinization			Complete	Liquid urine	++
	Chemical oxidation			Complete	Liquid urine	++
	Biological nitrification			Complete	Liquid urine	N.I.
Urine dewatering						
	Membrane	Reverse osmosis		Complete	Liquid UDFP	– –
		Forward osmosis		Complete	Liquid UDFP	– –
		Nanofiltration		Complete	Liquid UDFP	– –
		Membrane distillation[c]		Complete	Liquid UDFP	++[b]
				P, K	Liquid UDFP	++[b]
		Electrodialysis		N, P		++
	Evaporation/ distillation			Complete	Liquid UDFP	++
					Solid UDFP	++
	Freezing			Complete	Liquid UDFP	N.I.
Nutrient extraction						
	Stripping-absorption	Air stripping		N	Liquid UDFP	++[b]
		Membrane stripping		N	Liquid UDFP	++
	Adsorption	Direct use		N	Solid UDFP	–
				P	Solid UDFP	–
				part of N, P	Solid UDFP	–
				P, K	Solid UDFP	–
		Alkaline desorption		N	Liquid UDFP	++
		Acidic desorption		N	Liquid UDFP	N.I.
				P	Liquid UDFP	N.I.
				part of N, P	Liquid UDFP	N.I.
				P, K	Liquid UDFP	N.I.
	Chemical precipitation	Calcium phosphate		P	Solid UDFP	N.I.
		Struvite		part of N, P	Solid UDFP	N.I.
		Struvite-K		P, K	Solid UDFP	N.I.
		Filtration/centrifugation separation	Direct use	P	Solid UDFP	– –
				part of N, P	Solid UDFP	– –
				P, K	Solid UDFP	– –

(Continued on following page)

TABLE 2 (*Continued*) An overall assessment of pathogens inactivation in urine treatment technologies and the related UDFPs.

Technology		Nutrient recovered	UDFP	Inactivation[a]
	Drying	P	Solid UDFP	++[d]
		part of N, P	Solid UDFP	+
		P, K	Solid UDFP	++
	Struvite decomposition and reuse	N	Liquid UDFP	++

[a]The symbol + means that pathogens can be positively inactivated while – presents the opposite effect. Higher number of symbols indicates strong effect. NI means none information.
[b]Treatment with heating or at alkaline pH.
[c]Membrane distillation can concentrate P and K or N, P and K, respectively, determined by the operation conditions.
[d]Struvite can only be dried at temperature <35°C while struvite-K and other phosphate salts can be dried at higher temperature.

Decomposition of struvite (Eq. 1) can release ammonia which can be recovered through absorption in water or acid solution and the solid decomposition product can be employed to remove ammonia from urine through the re-precipitation of struvite (Eq. 2) (Chen et al., 2020). The decomposition was commonly conducted in ranges of 100–150°C using calcination (Huang et al., 2016), ultrasound radiation (Huang et al., 2017), and microwave radiation (Chen et al., 2020), the latter two processes of which were even carried out in alkaline solution containing NaOH. High temperature and combination of alkaline condition would probably inactivate pathogens in struvite, thus promising clean products of solid struvite and liquid ammonia-rich solution.

$$MgNH_4PO_4 \cdot 6H_2O(s) \rightarrow MgHPO_4(s) + NH_3(g) + 6H_2O \quad (1)$$

$$MgHPO_4(s) + NH_3(aq) + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O(s) \quad (2)$$

3.5 An overall assessment

Table 2 summarizes the overall assessment of pathogens inactivation in different urine treatment processes. Storage for 2–6 months was considered as an effective pretreatment technology to inactive pathogens when urine was used as liquid fertilizer (Schonning, 2001; Jönsson et al., 2004; World Health Organization (WHO), 2006). Urine dewatering and dehydration technologies are developed to obtain concentrated liquid fertilizer or solid fertilizer. Pathogens could be retained in concentrated urine fertilizer by membrane treatment using reverse osmosis, forward osmosis, and nanofiltration. This would bring strong negative effect on pathogens inactivation. However, electrodialysis may effectively remove pathogens because concentrated liquid fertilizer is obtained in the permeate side and pathogens are retained in the urine feed side. Moreover, membrane distillation should have strong positive effect on inactivating pathogens in concentrated urine fertilizer because of alkalinization or heating of urine feed. Evaporation under heating or distillation will probably yield sterile concentrated liquid or solid fertilizer products because high temperature is effective for pathogens inactivation.

Urine stabilization is commonly essential before urine dewatering to prevent loss of ammonia due to urea hydrolysis. Alkalinization can be used to enhance the pathogens inactivation as a pretreatment process, which may be particularly important for dewatering technologies without inactivation effect such as reverse osmosis, forward osmosis, nanofiltration and freezing. Pathogens would also be effectively inactivated by chemical oxidation. However, the effect of chemical oxidation on the characteristics of UDFPs is unclear. Moreover, it is also unclear whether acidification or biological nitrification can inactivate pathogens in urine. Thus, these two technologies would be suitable for urine stabilization before urine dewatering through membrane distillation, electrodialysis, evaporation and distillation while may be unsuitable prior to urine dewatering using reverse osmosis, forward osmosis, nanofiltration and freezing.

Absorption of ammonia by water or acid solution following stripping can recover N and yield liquid ammonia fertilizer. Air stripping with heating or at alkaline pH and membrane stripping would probably have strong positive effect on pathogens inactivation. Adsorption can recover specific nutrients from urine determined by the adsorbents' characteristics. However, direct use of the solid nutrients-enriched adsorbents in agriculture may pose microbial risks because pathogens may also be adsorbed on the UDFP. Alkaline desorption of nutrient from the solid UDFP to yield liquid fertilizer may be a promising post-treatment process to reduce the microbial risks. Nonetheless, alkaline desorption may be only suitable for ammonia recovery from urine. None information could be obtained on the pathogens inactivation through acidic desorption despite this post-treatment process can recover N, P and K in liquid UDFP. There lacks understanding on the inactivation of pathogens in the chemical precipitations of

calcium phosphate, struvite, and struvite-K. However, the following filtration or centrifugation separation of precipitates from urine would retain pathogens in the solid UDFP. This may pose strong negative effect on pathogens inactivation in UDFP. Post-treatment through drying with heating could effectively inactivate pathogens in the solid UDFP. Moreover, recovery of total ammonia in urine through recycling use of struvite precipitation and decomposition may also yield sterile liquid ammonia fertilizer because of struvite decomposition with heating and alkaline treatment.

Combination of different processes is usually essential to extract N, P and K from urine. Adsorption and precipitation was combined to yielding solid N-P enriched UDFP (Lind et al., 2000; Ban and Dave, 2004; Ganrot et al., 2007a; Ganrot et al., 2008; Xu S. et al., 2015; Zheng et al., 2018). This combined processes would have negative effect on pathogens inactivation because each separated process has negative effect. Moreover, precipitation of struvite and stripping-absorption can be combined to recover N and P producing liquid ammonia fertilizer and solid struvite. Pathogens in the liquid ammonia fertilizer would be reduced because of heating and alkaline treatment of urine. However, the pathogens in struvite were determined by the order in the combination. Pathogens in urine can be inactivated in the stripping process. Thus, struvite obtained in a precipitation process following the stripping (Pradhan et al., 2017) would have lower contents of pathogens than that yielded in a precipitation process followed by stripping(Antonini et al., 2011; Morales et al., 2013; Wei et al., 2018; Tao et al., 2019; Yang et al., 2022; Zhang et al., 2022). However, pathogens in solid product obtained in combined processes of stripping and precipitation of struvite-K may be inactivated because the operating pH in this precipitation was approximately 11 which benefits pathogens inactivation (Xu et al., 2017). This indicates that more attention should be paid to the interaction effects of processes combination on pathogens inactivation in further studies.

It should be noted that this study has not reviewed the treatment technologies which are developed to remove pollutants or recover energy from urine. Biological processes including nitrification-denitrification (Udert et al., 2003d), partial nitrification-denitrification (Yao et al., 2017) and nitritation-anammox (Buergmann et al., 2011) can remove nitrogen from urine. Microbial electrolysis cell can remove organics in urine (Boggs et al., 2009; Barbosa et al., 2018; Barbosa et al., 2019). The removal of N and organics has been examined through electrochemical oxidation processes (Amstutz et al., 2012; Dbira et al., 2015; Zoellig et al., 2015; Zollig et al., 2017; Cotillas et al., 2018a; Dbira et al., 2019). Energy production has been achieved *via* microbial fuel cells (You et al., 2016; Salar-Garcia et al., 2017; Gajda et al., 2019; Sabin et al., 2022). Advanced oxidation processes including chemical oxidation (Sun et al., 2018; Li et al., 2022), photocatalytic oxidation (Zhang et al., 2016), and electrochemical oxidation (Antonin et al., 2015; Cotillas et al., 2018b) have been developed to remove micropollutants. Pathogens inactivation in these technologies has not been discussed in this review. Microbial risks in UDFPs should be paid more attention when these technologies are combined with nutrient recovery technologies.

In addition, only the pathogens inactivation in storage and alkaline stabilization has been experimentally examined. Most of the cases were assessed based on publications about their effects on pathogens inactivation in other fields rather than in urine treatment. Moreover, none information on the pathogens inactivation could be obtained when urine was treated using acidic stabilization, stabilization *via* biological nitrification, dewatering *via* freezing or desorption after nutrient adsorption. This means that systematic and meticulous research work is urgently needed to provide technical support for better and safe development of the concept of urine source separation. This is particularly important for achieving U.N. SDGs in developing countries and regions lacking sanitation.

4 Outlook on microbial risk management of urine source separation

Disinfection of UDFPs and agricultural products is essential if recovery technology can't effectively inactivate pathogens in urine and the fertilizer product. Conventional technologies including high-temperature sterilization, ultraviolet disinfection and chemical oxidation disinfection have been proved to be effective for product disinfection (US Centers for Disease Control and Prevention, 2008). Cooking agricultural products before eating will be preferred to reduce the microbial risk by consumers. However, few publications reported the disinfection of UDFPs. Due care should be taken if these disinfection technologies would affect the fertilizer characteristics in further studies.

Moreover, it is essential to set standards on pathogen limitations in UDFPs and technical standards from the aspect of microbial risk management. The related standards lacks despite the concept of urine source separation has been proposed for nearly 30 years. The WHO guidelines recommends that the number of *E. coli* in water is less than 10^4/100 ml in the reuse of water in drip irrigation (World Health Organization (WHO), 2006). The Environmental Protection Agency regulations suggest that the number of *E. coli* should be less than 10^2/100 ml in the reused water (Environmental Protection Agency, 2012). The maximum allowable concentration of bacteria is 1000 CFU/g in recycled organic waste fertilizer products following the European Animal By-Products Regulation (The European Parliament and of the Council, 2002). These regulations may provide references for

UDFPs which are also used for agriculture. However, this has to be addressed in further studies.

Effective inactivation of pathogens in UDFPs would reduce the microbial risk to farmworkers and consumers of agricultural products. However, urine collection and transportation can also bring health risk to users. It is commonly assumed that the direct accidental ingestion of urine was 1 ml per case in recycling of urine in agriculture (Hoglund et al., 2002; Ahmed et al., 2017). Non-etheless, a recent study found that indirect ingestion also played a role in the exposure of urine collection users to urine (Bischel et al., 2019). The indirect ingestion refers to hand to mouth contacts with pathogens when hands, skin and clothes are contaminated and then hands bring pathogens to mouth. Furthermore, operating struvite precipitation reactor also resulted in health risk due to direct and indirect contacts with pathogens-carrying substances (Bischel et al., 2019). This indicates that microbial risk in urine source separation should consider all possible exposure pathways besides the exposure to UDFPs. Therefore, it is recommended that personal protective equipment (facemasks, gloves, work clothes, etc.) is essential to reduce the exposure of urine collection users and farmworkers to urine and UDFPs.

5 Conclusion

Pathogens in source-separated urine can be introduced through two approaches including excretion with urine and fecal contamination. A summary on bacteria and viruses detected in urine confirms that urine is not sterile. Then, this study reviewed and evaluated pathogens inactivation in urine treatment technologies classified based on their UDFPs. Storage and alkaline stabilization have experimentally proved effective effects on pathogens inactivation of urine. Technologies including membrane distillation, electrodialysis, evaporation, distillation, air stripping, and membrane stripping should have positive effect on pathogens inactivation of UDFPs because alkaline treatment, high temperature and membrane separation in these technologies could reduce pathogens. However, pathogens might be concentrated when urine is dewatered using reverse osmosis, forward osmosis, and nanofiltration. Moreover, adsorption and precipitates separation using filtration and centrifugation may also present negative effect on pathogens inactivation in UDFPs. Non-etheless, post treatment through alkaline desorption, precipitates drying, and struvite decomposition and recycle would benefit the disinfection of UDFPs. Finally, an overall assessment was carried out to show a clear map of the connection of technologies and the pathogens inactivation in UDFPs. Based on the assessment, this review discussed the lack of experimental studies on pathogens inactivation in urine treatment and presented outlooks on development of new urine treatment technology and management of microbial risk in urine recycling in agriculture.

Author contributions

KX: investigation and writing—original draft. JLu and HL: investigation, writing—review and editing. JLi: conceptualization, writing—review and editing, funding acquisition. SC: conceptualization, writing—review and editing. MZ: writing—review and editing. CW: conceptualization.

Funding

This study was supported by the National Key Research and Development Plan of China (No. 2020YFD1100102).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.1056019/full#supplementary-material

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