

Chapter 2

Case of Venice (Italy)



Patrizia Ragazzo and Nicoletta Chiucchini



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2.1 INTRODUCTION

2.1.1 The initial context

This project started up in 2005 with the aim of identifying a valid alternative to chlorine in the disinfection of wastewater. Given its tendency to form disinfectant byproducts (DBPs), as early as 2004 Veneto Region Authority had expressed the intention to proceed with extensive chlorine prohibition in wastewater disinfection. The need had therefore emerged to quickly identify a disinfection alternative that, by ensuring bacterial inactivation performance similar to that of chlorine, did not involve byproduct formation. In reviewing the alternatives

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available at the time, peracetic acid (PAA) and UV irradiation constituted the main solutions capable of being adopted. However, since the effectiveness of UV treatment is highly influenced by the quality characteristics of effluent and moreover implies high operating and capital costs while PAA entails other noteworthy disadvantages (qualitative or economic), we did not consider either of them to be adequate solutions.

Hence, we laid out two main lines of research aimed at identifying alternatives, one regarding the circumstances of potentially maintaining chlorine use and the other focused on studying the applicability and reliability of a new chemical (performic acid, PFA), produced by the Finnish company Kemira, proposed as a disinfectant in wastewater.

The suitability of PFA in wastewater disinfection was preliminarily assessed at our laboratory by carrying out batch tests on actual matrices consisting of secondary wastewater effluent, and by verifying the effectiveness of PFA on fecal coliforms *Escherichia coli* (*E. coli*) and enterococci at doses and contact times of 1–5 mg/L and 10–60 min, respectively. Based on the encouraging results shown for PFA effectiveness versus those of more traditional disinfectants, such as PAA and chlorine (Ragazzo *et al.*, 2007), we initiated the first full-scale experiments, the first of which was carried out in winter 2005 and summer 2006 at the *Caorle* municipal wastewater treatment plant (120,000 population equivalent).

These studies were conducted by using both the first production system prototype (Figure 54a and b) and the more heavily automated pilot *Hyproform* system (Figure 54c and d). As part of these investigations, the effectiveness and potential impacts were tested on a number of key effluent quality parameters (e.g., pH, total organic carbon, other chemical and physical parameters) as well as on the indirect ecotoxicity introduced into the environment.

Further study phases examined other WWTPs (Table 23) and years (2011–2018), with the aim of consolidating the knowledge of PFA with respect to disinfection efficacy and oxidative power on organic matter, in addition to direct and indirect toxicity induced by the doses applied, for potential products generated from its decomposition.

Throughout the experimental campaigns on PFA, specific studies were conducted in parallel within the same WWTPs for comparison with other reference wastewater disinfectants, such as chlorine and PAA.

The full-scale studies demonstrated that PFA is an effective and reliable disinfectant for wastewater treatment applications (Ragazzo *et al.*, 2013), and its effectiveness is comparable and greater than that of chlorine hypochlorite (HYP) and PAA (Ragazzo *et al.*, 2020).

This chapter will report some of the main results obtained, in compliance with the confidentiality agreements set forth by the scientific journals that have published our results. A certain amount of the data has been reprocessed in order to focus on specific aspects of PFA disinfection, while other data are presented here for the first time.

2.1.2 Disinfection methods in VERITAS

Ours is a public multi-utility managing 36 municipalities covering the integrated water cycle, servicing a total of some 800,000 inhabitants across a territory visited every year by millions of tourists. Drinking water is produced from both ground (81%) and surface (19%) sources. Wastewater (95 Mm³/years) is treated in 38 treatment plants (WWTPs) for a total capacity exceeding 1.2 million population equivalent (p.e.). Eleven WWTPs have been designed to serve between 10,000 and 400,000 p.e., six of which encompass bathing locations along the Adriatic coast: Jesolo, Caorle, Eraclea, Cavallino, Lido di Venezia, Chioggia (capacity range: 32,000–160,000 p.e.). The sensitivity of this territory due to the Venice Lagoon and coastal bathing zones requires special attention to wastewater management. For this reason, stringent regulations on both discharged loads and WWTP treatment requirements were drawn up beginning in the early 1970s (D.P.R. 962/1973). The ban on chlorine use in wastewater disinfection is part of these restrictions. The first prohibition dates back to the early 2000s (D.M. 1999) and referred to effluents directly or indirectly discharging into the Venice Lagoon. This interdiction was then extended to the entire region 14 years later, in 2013 (Deliberation no. 107/2009, Veneto Region, 2009). This context prompted the conversion of disinfection treatments in the region toward alternatives, at the time considered to be less impactful. In the case of Veritas, the initial conversions were to the UV technology at the Fusina (400,000 p.e.) and Campalto (130,000 p.e.) WWTPs, as part of an integrated project to adapt infrastructure to safeguard the Venice Lagoon. By extension later on, other WWTP disinfection treatments were upgraded, by adopting the only chemical alternative to chlorine available during those years (2000–2010). To date, 13 installations offer active disinfection: three use UV technologies (130,000–400,000 p.e.), eight disinfect with PAA (2500–105,000 p.e.), and two disinfect with PFA (32000–160,000 p.e.).

2.2 MATERIALS AND METHODS

2.2.1 Studies and the WWTPs

All studies on PFA, from 2005 to 2018, were carried out with both laboratory tests and full-scale experiments. For this purpose, three coastal WWTPs – i.e., Jesolo (160,000 p.e.), Caorle (120,000 p.e.) and Eraclea (32,000 p.e.) – and the remote San Dona'di Piave plant (45,000 p.e.) were used. All plants provided a conventional sequence of treatment (primary clarification, active sludge oxidation-nitrification and denitrification, secondary settling) for municipal wastewater, collected primarily by combined (60%) sewage systems, with insignificant industrial discharges. The effluents were only disinfected during summer in order to respect the national *E. coli* regulation limit of 5000 CFU/100 mL.

The effectiveness of the disinfectants was tested against fecal coliform (FC), *E. coli* (EC) and the more resistant fecal enterococci (FE). Table 23 provides an overview of all the main investigations undertaken.

All the plants were equipped with traditional chicane disinfection channels; moreover, the doses set to guarantee the compliance limit are flow-paced and maintained to be stable through a standardized management/control system.

Performic acid disinfection solution was produced on-site, just before dose application, with the two systems provided by Kemira Oyj: Hyproform (2005/2006) and Desinfix (starting in 2011). Besides the technical and safety aspects, which were greatly improved in the final unit, the major difference among the systems lies in the compositions of the equilibrium solutions produced, with higher oxidant concentration ranges in the Desinfix (PFA 12–15% w/w and H₂O₂ 18–20% w/w), with respect to Hyproform (herein denoted PFA-HP) (PFA 8–10% w/w and H₂O₂ 11–13% w/w). A detailed technical description of the systems has been reported in Ragazzo *et al.* (2013).

For the other two disinfectants used here as references, commercial solutions with an active substance nominal titer of 12% w/w for hypochlorite and 15% w/w for PAA were applied.

2.2.2 Sampling methods

Laboratory tests focused on bacterial inactivation over time at different disinfectant doses. For this purpose, samples collected at the WWTP outlet were taken to the laboratory for processing through batch trials at various disinfectant concentrations; upon each test, the samples were quenched for oxidant residual and analyzed for bacterial concentrations according to the method outlined in Table 24. Other batch trials were performed in order to correlate the carbon-based disinfectants PFA and PAA with the organic carbon increase, especially its biodegradable component. Deionized water solutions corresponding to disinfected water, ranging from 0.5 to 10 mg/L, were tested and then total organic carbon (TOC), and chemical and biochemical oxygen demand (COD, BOD₅) were analyzed according to the methods shown in Table 24.

During the full-scale studies, the physicochemical parameters (pH, total suspended solids (TSS), COD, nitrite, ammonia, etc.) at the disinfection inlet were evaluated, along with disinfectant effectiveness by means of FC, EC and FE fecal indicators. In some cases, the EC and FE concentrations at various retention times along the disinfection channel were also determined.

The PFA qualitative impact on organic compounds was assessed by monitoring TOC and formate (FA) concentration, both before and after PFA application. Ecotoxicity impacts were assessed using the *Vibrio* fishery and *Daphnia* Magna tests.

All analyses were conducted in the laboratory on composite wastewater samples, obtained by collecting in the same bottles equal aliquots of effluents at different time

intervals (every 2–3 hours) in relation to the flow rate; sodium thiosulfate had been previously added to the bottles so as to reduce oxidant residuals.

The disinfectant solution was periodically controlled for the concentration of active substances by titration with cerium ammonium sulfate and sodium thiosulfate for PFA-PAA (Greenspan & MacKellar, 1948) and by iodometric titration with sodium thiosulfate for chlorine (UN EN 901:2007). The oxidant residuals at the disinfection outlet were controlled on a daily basis by means of the DPD colorimetric method, according to APHA 4500-Cl G, with and without the prior addition of catalase enzyme to account for the H₂O₂ contribution in the case of peracids. Table 24 summarizes the methods employed at both the full and laboratory scales.

2.2.3 Data processing

The level of effectiveness was analyzed using the non-parametric Kruskal–Wallis test. The ANCOVA test was applied to evaluate the effects of disinfectants, adjusted for doses and contact time. The t-Student and non-parametric Wilcoxon tests were used for comparisons of quality pair data, respectively for normal and non-normal distributions (based on the Shapiro–Wilks test). Associations among variables were assessed by the Spearman rank test. Bacterial inactivation results were modeled using Hom and S-Model (Luukkonen *et al.*, 2015); kinetics parameter values were obtained by means of non-linear regression run with the Microsoft Excel Solver-function GRG nonlinear.

2.3 RESULTS

2.3.1 Experimental set-up

The first full-scale results on PFA effectiveness were obtained in 2005–2006 by using the prototype and pilot production system (Hyproform, denoted here as PFA-HP). From 2011 onwards, all PFA studies were carried out using increasingly more advanced production systems, culminating in what became the definitive Desinfix production system (denoted here as PFA). Moreover, in those plants where HYP and PAA were routinely used, specific management and monitoring criteria were implemented, in addition to adapting frequency and control type, in order to obtain effectiveness data useful in comparisons. Table 25 lists the installations and operational conditions of the full-scale experiments for all disinfectants presented and discussed in this section. All plants serve seaside locations and are characterized by high seasonal and daily load variations; retention time in the disinfection channel, which is consistently adequate at the Caorle and Jesolo WWTs, was more critical at Eraclea, where values shorter than 10 min were often recorded. Disinfectant doses, previously individuated to meet the 5000 CFU/100 mL *E. coli* target at the outlet, were generally maintained.

2.3.2 Effectiveness

Table 26 provides the median and range of variation of the main qualitative characteristics of the secondary effluents entering the disinfection reactor for each disinfectant applied (Kruskal–Wallis test). Since these effluents stem from the biological oxidation process, the main physicochemical parameters capable of impacting with disinfectants are: pH, total suspended solids, nitrous and ammonium nitrogen, organic compounds (COD, BOD₅), and the bacterial concentration itself.

Qualitative parameter variations were similar to one another except for ammonia, which was high particularly in the case of disinfection with HYP and PFA (nitrite was always below the detection limit of the analytical method: 0.02 mg/L). Ammonia is reported not to interfere with PAA and PFA; with chlorine, it was determined that the disinfection mainly occurred by monochloramine (Ragazzo *et al.*, 2020). Total suspended solids and COD were always below the values reported as interfering with disinfectants; for pH, only PFA could have interfered with the neutral values (Ragazzo *et al.*, 2020). Bacterial concentrations entering the disinfection channel were comparable ($p > 0.05$, Kruskal–Wallis test), with the exception of fecal coliforms and *E. coli* under PFA-HP disinfection (where lower values were recorded compared to other disinfectants, $p < 0.001$); for PFA-HP, the Spearman test found weak correlations with the corresponding bacterial reduction ($R \sim 0.52$ – 0.60 for fecal coliforms and *E. coli* respectively, $p < 0.01$).

Weak correlations were also found for PAA and PFA, respectively, between the enterococci and *E. coli* detected at the inlet and corresponding values at the outlet. Only in the case of PFA-HP was a correlation found, albeit weak, between fecal coliforms measured at the outlet and TSS values at the inlet ($R \sim 0.52$, $p < 0.01$). The results obtained at full scale from 2005 to 2011 are given in Table 27; data are provided in terms of statistical comparison among the bacterial inactivation achieved by the various disinfectants, each one under the respective operating conditions of dose and contact time (via the Kruskal–Wallis test).

PFA appears in both forms: the oldest disinfection solution with a PFA of around 9% w/w (PFA-HP) and the new solution (PFA) containing a PFA of approximately 14% w/w. At contact times between 10 and 20 min, at doses that were one-half and one-third of those of PAA and HYP respectively, PFA achieved the highest inactivation of all fecal indicators, while PFA-HP and PAA the lowest against *E. coli* and enterococci, respectively. With less constraining contact times however, the PFA-HP achieved *E. coli* reductions comparable to those with HYP and PAA, as well as an enterococci inactivation similar to that of HYP; put otherwise, it performs better than or comparable with the other chemical disinfectants.

Under very limited retention times, PFA achieved *E. coli* and enterococci inactivation consistently greater than that of PAA over all time intervals, with

enterococci reductions being comparable to those reached by chlorine at 30-plus minutes. Figure 55 provides the exceedance probability plot of disinfectants from 10 to 20 minutes.

The ANCOVA test was applied to these same results to compare the disinfectant performance in taking into account the contribution of doses applied, thus evaluating the actual effectiveness of the disinfectants. The order of effectiveness indicated by this test is summarized in Table 28.

Bacterial inactivation was fitted with the Homs and S bacterial models to compare PFA and PAA; the range of parameters estimated is reported in Table 29. Fecal indicator inactivation has been well described by both models (R^2 always above 0.91) at doses between 0.5 and 1.3 mg/L for PFA (mainly at the full scale) and 1–3 mg/L for PAA (mainly at the laboratory scale). Hom's model shows the limited importance of disinfectant concentrations, except for PAA, against enterococci ('n' values greater than the others), as well as the importance of retention time, which increases with bacterial resistance ('m' values greater for Enterococci than *E. coli*) and the disinfectant strength decrease ('m' values less for PFA than PAA). These findings are in agreement with results reported by Luukkonen *et al.* (2015). The K values for both models further confirmed the higher sensitivity of *E. coli* than enterococci to disinfection (values for *E. coli* greater than those for enterococci).

2.3.3 Reuse goal

Results obtained during experiments at the full scale (2005–2011) were analyzed to verify the extent to which the disinfection systems, set for compliance at the current target (5000 *E. coli* CFU/100 mL), were able to meet more ambitious goals. For the four disinfection systems tested, Table 30 reports the percentage of values at the disinfection outlet capable of respecting various limits: from the targets to be respected (the highest value, representing the general limit that effluents are to respect for discharge into surface waters) to the other two more restrictive targets, used as conservative levels to guarantee compliance. In the case of *E. coli*, 1000 CFU/100 mL offers a guideline value by the WHO for agricultural reuse in countries without drought issues; 10 CFU/100 mL must be respected in Italy for this same kind of reuse (D.M. 185/2003).

The ability to comply with the various limits can be summarized in the following order: HYP > PFA-HP > PAA with fecal coliforms; PFA > HYP~PFA-HP > PAA against *E. coli* and enterococci. Moreover, the PFA constantly met the 1000 *E. coli* WHO guideline value and yielded the best performance for the other targets as well.

The ANCOVA model applied to the results discussed above, including doses and contact times (covariates), provides a forecast of disinfectant effectiveness adjusted for both covariates; Table 31 shows how the various systems can guarantee a low level of microbiological risk in the effluent. Among all the bacterial indicators,

PFA is supposed to be the most effective in guaranteeing a low level of microbiological risk, especially with enterococci; the values guaranteed at the discharge outlet by PAA and PFA are respectively 1.5 and 0.2 times those of chlorine. In other words, the microbiological values in the effluent guaranteed by PAA are 7–9 times higher than those guaranteed by PFA (for Fecal coli to *E. coli*, respectively).

2.3.4 Compliance over time

Subsequent to these studies, PFA disinfection technology was adopted in all the WWTPs where the experiments were conducted (Caorle, Eraclea, San Donà e Jesolo). For the several contact time intervals of WWTPs over the six-year period (2013–2018), [Table 32](#) reports the number of controls performed, the corresponding doses applied, and the percentage of results able to meet 1000 CFU/100 mL and the two additional, more ambitious *E. coli* targets. The physicochemical characteristics of the effluent were those typical of the WWTPs: some key parameters are indicated in terms of average, standard deviation and maximum value (in brackets) in [Table 33](#).

The *E. coli* goal (5000 CFU/100 mL) was respected at a high level of confidence (1000 CFU/100 mL) in 99% of controls (1000 CFU/100 mL). When taking into consideration all WWTPs, the PFA doses ranged from a minimum of 0.4 to a maximum of 1.1 mg/L, and retention times in the disinfection channel were typical of each installation, that is relatively high for Caorle and San Donà (52 and 27 min on average, respectively) and quite low at Jesolo and Eraclea (20 and 10 min, respectively). The few values that exceeded 1000 *E. coli* CFU/100 mL were recorded at the Eraclea WWTP during high season. The *E. coli* limit established for Italian agricultural reuse (10 CFU/100 mL) was met in less than 20% of cases across all installations except for San Donà, where this limit was met in 58% of cases.

In the two-year period 2016–2017, PFA residuals at the disinfection outlet were measured three times a day at each installation to monitor the decomposition of oxidant residuals. In 97% of the measurements, the PFA dose was below 0.8 mg/L (total average: 0.6 mg/L), and retention times were shorter than 20 and 40 min in 53 and 74% of the cases, respectively. The average retention times in the disinfection reactors were ranked as follows: Caorle > S. Donà > Jesolo > Eraclea, where the extremes were respectively 52 and 10 min.

The trend in PFA residuals at the disinfection outlet was significantly correlated with retention times in the disinfection channels (R Spearman 0.66 $p < 0.01$), regardless of the matrix variation and uncertainty due to in-field measurements and low concentrations. Unlike PFA, H₂O₂ was consumed rapidly and, regardless of contact time, with residuals ranging from 14 to 10% of doses applied at Caorle and Eraclea, respectively.

Figure 56 shows the average residual concentrations of PFA at the four WWTP disinfection outlets vs. their average contact times.

2.3.5 Quality impacts

Several works in the literature have referred to the low propensity of PFA and PAA to form disinfection byproducts. In reference to other authors, Luukkonen and Pehkonen (2017) postulated, for example, that one of the main advantages of PAA over free chlorine or ozone is the reduced probability of forming DBPs and moreover that PFA, though less widely studied in terms of byproduct formation, does form DBPs similar to or even slightly lower than PAA.

Similarly, several pieces of evidence point to the impacts of PAA on the organic substance of the effluent. Both COD and TOC increase as a result of peracid dosing, and it is possible to calculate the theoretical increase based on the peracid equilibrium composition (Luukkonen *et al.*, 2014).

The impact of PFA on the quality characteristics of secondary effluent was examined several times during our experiments, between 2005 and 2017. Initial studies examined the variation, due to the doses applied, in key quality parameters such as TOC, BOD₅ and pH (Ragazzo *et al.*, 2013). Subsequent research on the impacts of PFA have focused on the potential cytotoxic, genotoxic and mutagenic effects of PFA (Ragazzo *et al.*, 2017), as well as on certain implications (linked to its use), such as the potential direct toxicity on the environment and the ability to oxidize some of the categories of organic compounds more recalcitrant to oxidation (Ragazzo *et al.*, 2020).

Given the greater ability of PFA, in comparison with PAA, to inactivate the most resistant fecal indicators (enterococci), the ability of PFA to oxidize organic substances has been constantly assessed, either directly on generic organic substances or through indirect testing (Ragazzo *et al.*, 2013, 2020).

No pH variation or organic matter oxidation was detected at doses applied at the full-scale (Ragazzo *et al.*, 2007, 2013). This finding was subsequently confirmed by additional literature reporting a weak ability for PFA to oxidize pharmaceutical compounds, endocrine disruptors and bisphenol-A (Gagnon *et al.*, 2008; Luukkonen *et al.*, 2015).

For each interval of contact time in the disinfection reactor, Tables 34 and 35 compare TOC and formate by using the Student's and Wilcoxon tests, respectively.

The comparison is drawn between values calculated at the dosage point T_0 (obtained by adding the stoichiometric values derived from the applied PFA dosage to the measured values at the inlet) and those measured at the disinfection reactor outlet.

All values measured at the discharge outlet corresponded to the expected theoretical values ($p > 0.05$), with the exception of two time intervals for TOC, during which the values increased from the disinfection reactor inlet to outlet; this variation however was less than or equal to the uncertainty of the analytical

method (5–10%). These results suggest a poor ability of PFA to oxidize the organic substance, even the simplest, co-generated and coexistent with the disinfectant itself, in addition to a potentially low tendency to form byproducts.

Specific laboratory tests carried out to correlate the carbon-based disinfectants PFA-HP and PAA with the biodegradability of corresponding residual molecules showed a poor biodegradability in terms of the BOD₅ of formate (co-present and intermediate decomposition product of PFA) with respect to acetate (co-present and intermediate product of decomposition of PAA). Table 36 correlates PFA-HP and PAA doses (applied or simulated) with the several corresponding components of measured organic carbon. In the case of the PFA solutions being used today, these components are reduced to 0.7, 1.0 and 0.3 mg/L for TOC, COD and BOD₅, respectively.

The toxic effect of PFA was measured in relation to the potential byproducts formed by its reaction with organic and inorganic substances present in the matrix. Toxicity was evaluated both in terms of eco-toxicity induced on *Vibrio* Fishery and *Daphnia Magna* (at the doses applied at full-scale, averaging around 1 mg/L, variation range: 0.4–2.4 mg/L) and in terms of mutagenic, cytotoxic and genotoxic potential, by measuring short-term effects on bacterial, plant and mammalian target cells (doses ranging from 0.6 to 1.5 mg/L). In no cases were any depression effects actually recorded on *Vibrio* Fishery light emission $\geq 50\%$ (Ragazzo *et al.*, 2013) or on *Daphnia Magna* mobility (zero effect consistently reported). Light suppression, which varied from a minimum of 0% to a maximum of 43%, did not differ from the typical effluent values in the absence of disinfection treatment ($p > 0.05$).

The mutagenic and genotoxic effects were always negative in all *in-vitro* tests, performed on both disinfected and non-disinfected effluents. In tests with *Allium cepa* however, in some samples of unconcentrated wastewater, treatment with PFA did induce a slight increase in the frequencies of the micronucleus in root cells, uncorrelated with the disinfectant doses (Ragazzo *et al.*, 2017).

2.3.6 Protection outcomes and economic evaluations

Effectiveness levels obtained for PFA and PAA in the studies performed from 2011 to 2017, when examined as a whole, allow some conclusions to be drawn on doses and costs required to guarantee, at least in theory, a suitable level of protection from microbiological risks in wastewater disinfection. For both peracids, the doses were set at the minimum value in order to guarantee stable compliance of 5000 CFU/100 mL *E. coli* at the disinfection outlet, which corresponds to respecting the microbiological targets at 20% of the guideline or limit values (Ragazzo *et al.*, 2020).

Table 37 summarizes the percentage of cases and corresponding operating conditions in which the two peracids did respect the conservative microbiological targets.

The 1000 CFU/100 mL *E. coli* and 400 CFU/100 mL enterococci targets were achieved by PAA-PFA in 92–99 and 48–88% of cases, respectively, at applied doses 2–3 times higher for PAA than PFA. In other words, PFA guaranteed higher compliance against *E. coli* despite being much more often under less favorable contact time conditions; given the rather similar operating conditions against enterococci, PFA provided a level of protection nearly double that of PAA.

Costs vary depending on different factors (allocation, type of WWTP and wastewater characteristics, microbial target, etc.); hence, definitive evaluations cannot be given. Nevertheless, some conclusions can be drawn based on the stability of results reported and discussed by Ragazzo *et al.* (2020). Even though the commercial costs of the PFA active substance are on average roughly 1.5 times those of PAA (€10.8/kg and €7.6/kg, respectively – values extracted from internal and literature data – Luukkonen *et al.*, 2015; Maffettone *et al.*, 2018), in all trials the reagent disinfection costs were higher for PAA compared to the on-site generated PFA, that is €0.018/m³ and €0.010/m³ on average, respectively.

Key points

- PFA technology appears to be a valid alternative to chlorine and moreover offers some decisive advantages over peracetic acid disinfection.
- Its undisputed efficacy against enterococci under all operating conditions tested is the main reason why we chose PFA as the disinfection technology for plants with effluent more directly connected to the Adriatic coast and bathing zones protection issues. We therefore regard this technology as a solution to be adopted more extensively in the near future.