Innovative and low-cost auto-sampler for SARS-CoV-2 in wastewater surveillance program

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ABSTRACT: The theory of wastewater-based epidemiology has been applied in SARS-CoV-2 near-source tracking, which provides public health authorities with an early warning system to COVID-19 outbreaks, currently a global public health crisis. An innovative and low-cost auto-sampler named MAD-AS is designed as an alternative to other traditional wastewater sampling methods that are unsuitable for the wastewater surveillance program due to various limitations. The small and reusable MAD-AS uses waterproof 3D-printed parts and an Arduino-operated control system. It can mitigate the clogging problem caused by the debris in the wastewater and automatically collect water samples in high temporal resolution. The design prototypes are tested in the laboratory utilising electrical conductivity (EC) solution to simulate virus concentration variation in the wastewater. The EC values of the collected samples are compared with the expected EC values using the time-weighted average approach. The conceptual design is preliminarily validated as the absolute differences of the results are smaller than 0.2mS/cm in most cases. A trial deployment to a wastewater treatment plant in Victoria, Australia, further proves the functionalities and waterproofness of the auto-sampler. Further field experiments in different environment and locations should be done to evaluate the reliability and accuracy of MAD-AS in different scenarios. It is believed that the MAD-AS auto-sampler not only works well for wastewater-based epidemiology but also can be used for stormwater and natural waterway sampling on large spatial and temporal scales.

1 INTRODUCTION

1.1 *Background*

The COVID-19 global pandemic caused by the novel coronavirus (SARS-CoV-2) has seriously triggered public health crisis in almost every country. More than 160 million people have been infected, and 3.3 million people have died from this novel coronavirus as of 14th May 2021(WHO, 2021). To suppress outbreaks and contain and control the spread of the respiratory disease, public health authorities use clinic testing on the individuals to identify people who have infected by the virus. Nevertheless, there are limitations to this invasive approach as it is expensive, time-consuming, and labour-intensive. Another disadvantage of clinic testing is that people only get tested after they are aware of the symptoms. But the people can be mobile source of infection before they develop symptoms and decide to get tested. The infected people can also be asymptomatic and therefore not get tested. As this approach has an inherent delay and is not efficient and prompt enough, alternative monitoring and detection approaches should be developed to build a responsive early-warning system for potential local outbreaks and community transmission. (Orive *et al.*, 2020)

The current clinic testing is typically taking sample swabs from throats and noses, but SARS-CoV-2 has also been found in the anal swab test and the infected patients' stool sample (Gupta *et al.*, 2020; Holshue *et al.*, 2020). The virus RNA can be detected in stool samples from symptomatic and even asymptomatic patients (Mirjalali *et al.*, 2020). In a study by Zheng *et al.* (2020) on the viral dynamics of SARS-CoV-2, the median duration of this virus in the stool sample is found to be 22 days, longer than the median duration in respiratory (18 days) and serum samples (16 days). Therefore, the researchers have found that it is feasible to detect SARS-CoV-2 shedding from human bodies, through the human faeces deposits and spit and snot delivered during bathing events, in the wastewater. It is in the theory of Wastewater-Based Epidemiology (Mao *et al.*, 2020).

Wastewater-Based Epidemiology (WBE) is an epidemiological tool that collects and assesses untreated wastewater from the treatment plant inlet. It has been used to provide near real-time and comprehensive health information, such as specific diseases, drug consumption, exposure to certain agents, and lifestyle consequences, of the entire population in the well-defined geographical wastewater catchment area (Lorenzo and Picó, 2019; Sims and Kasprzyk-Hordern, 2020). Research on the application of WBE in COVID-19 control and prevention had begun since the global pandemic started in 2020. The research focus has moved from collecting samples from wastewater treatment facilities that serve large populations (CDC, 2020; Wu *et al.*, 2020) to sampling from the upstream sewage pipeline network and community-level catchment areas, such as schools and residential facilities (Liu *et al.*, 2020). The latter is called near-source tracking (NST) and allows the detection and identification of small clusters or even infected individuals within smaller groups of population and smaller geographical area (Hassard *et al.*, 2021).

1.2 *Literature Review*

Selecting appropriate sampling methods is important to the effectiveness of COVID-19 near-source tracking. Grab sampling and composite sampling (manual and automated) are commonly used in wastewater surveillance and WBE (CDC, 2020). A new passive sampling technology has been recently tested and discussed (Schang *et al.*, 2020). The innovative and low-cost passive samplers have been widely used in the COVID-19 wastewater surveillance program across Victoria, Australia (DHHS Victoria, 2021). The existing sampling methods are well-established, and each has respective advantages comparing to the others. However, these methods also have corresponding problems when used in temporary and short-term deployment (Doriean *et al.*, 2019), which is typically required in near-source tracking. Therefore, the limitations and constraints of each sampling are evaluated in detail.

Grab Sampling. Manually taking water grab sample is the easiest sampling method. However, the sample taken only represents a single moment of an entire day. We may miss the important events and not capture the virus at this particular moment. Also, the workers may need to work in confined space (such as manholes) and the exposure to wastewater is a concerning OHS (occupational health and safety) problem. (CDC, 2020)

Manual Composite Sampling. Although multiple water grab samples are taken at different times of a day, the samples may still not be taken frequently enough to capture the virus. Also, frequent manual sampling requires more intensive and expensive labour input and enlarges OHS risks. (CDC, 2020; Schang *et al.*, 2020)

Automated Composite Sampling. The costly and not widely available automated samplers are usually used in the wastewater treatment plant instead of temporary and flexible deployment. They cannot be installed in the pipeline/manhole due to confined spaced and limited power access. Also, commercial automated samplers have strict limits on how many times they can collect before the sample storage is full. (Doriean *et al.*, 2019)

Passive Sampling. It is a qualitative method (if the virus existed) rather than a quantitative method (meaningful concentration of the virus). It only tells the presence or absence of the virus during the period of deployment. Therefore, we cannot determine how many infected people are in the catchment, rather just whether there is one or more. The passive samplers used in the SARS-CoV-2 in wastewater surveillance program in Victoria, Australia, are single-use and disposed of as biohazard waste after laboratory analysis. Intensive labour input is required for mass production. Additionally, the samplers are vulnerable to the clogging and blockage problem caused by debris in the wastewater, which prevents the sample from entering the sampler. (DHHS Victoria, 2021; Schang *et al.*, 2020)

The literature review also looked at stormwater and waterways sampling methods that could potentially be used for near-source tracking. The objective of sampling in natural water bodies is to understand and manage changes to sediment, nutrient, and contaminant concentrations in the aquatic environment, especially after storm events (Doriean *et al.*, 2019). The aforementioned sampling methods are also widely used in stormwater and natural water sampling with similar limitations, which make research challenging.

Surrogate Parameter. In addition to the commonly used techniques, some researchers use in-situ turbidity data loggers as a “surrogate” to predict suspended sediment concentration. (Doriean *et al.*, 2020; Kim and Furumai, 2013). However, there is no knowledge that if the presence/absence or concentrations of SARS-CoV-2 can be estimated based on any “surrogate” wastewater parameter, hence this approach is not feasible yet.

Peristaltic Pumping. Doriean *et al.* (2019) developed an inexpensive sampler that was operated by a peristaltic pump. The water sample was pumped into the sampler at a controlled flow rate, and the suspended solids would remain in the sampler body after sedimentation. Although the virus cannot settle in the sampler body like the suspended solids without electronic-charged filter paper (Ahmed *et al.*, 2020), this innovative and low-cost design using a peristaltic pump provides a new idea for spatially extensive water sampling requirements.

1.3 *Problem Definition*

In conclusion, the SARS-CoV-2 wastewater surveillance or more general Wastewater Based Epidemiology requires that the sample should be taken frequently and be taken flexibly on both large spatial and temporal scales. The wide-spread deployment can include the downstream treatment plant and the upstream sewer network. The present sampling methods without significant modification are not suitable sampling solutions for the scenarios

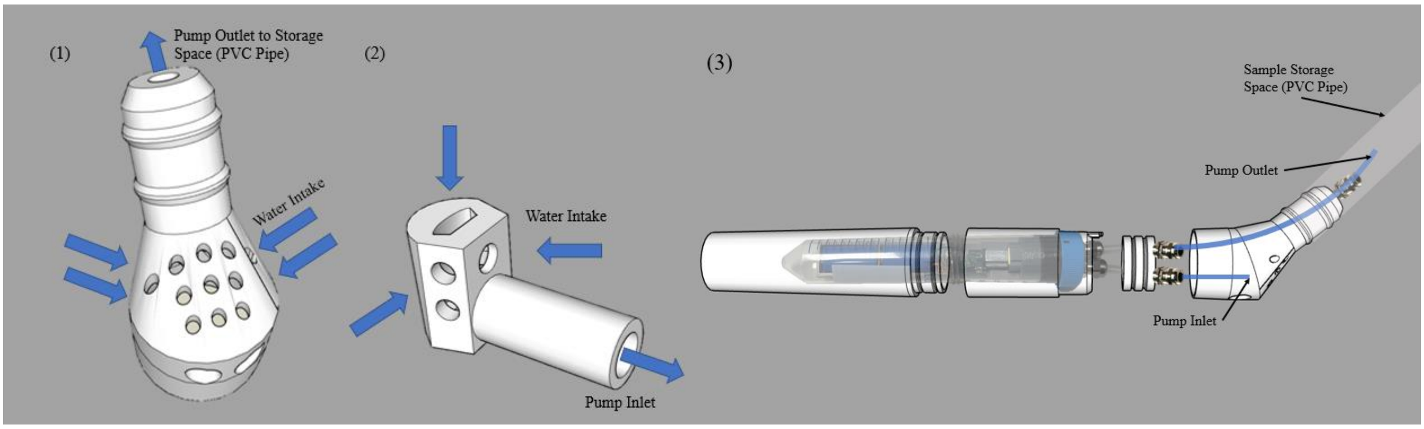

Figure 1. (1) 3D-printed case front. (2) 3D-printed filtering component to be installed on the pump inlet and mitigate clogging problem. (3) Assembly of the outer cases and PVC pipe. (Not to scale)

we are dealing with due to various drawbacks. For example, no suitable sampling devices can be flexibly deployed, and the workers may need to physically enter the confined space (such as manholes) and get exposed to wastewater. Therefore, an innovative sampler should be developed with the following features to overcome these problems.

The desired sampling method should be capable to automatically and frequently collect and store composite water sample. It needs to be cheap and easy to build, deploy, maintain, and retrieve with all building materials widely available. The sampler body should be small enough to be deployed in the confined space and minimise OHS risks and workers' exposure to wastewater. The sampler can work without an external power supply and can be reused after retrieval. While the ideal sampler is able to mitigate the blockage and clogging caused by the debris in the wastewater, it preferably can also be used for stormwater and natural waterway sampling.

This paper aims to present the development and preliminary testing (laboratory simulation and field trial) of an innovative auto-sampler (named MAD-AS) built based on a 3D-printed peristaltic pump (McCarthy *et al.*, 2021). The paper reviews how and how well the aforementioned features are achieved. Also, the testing examines how representative the collected wastewater sample is. Limitations of this auto-sampler and recommendations on further work are discussed as well.

2 METHODOLOGY

2.1 *Hardware Description*

The MAD-AS auto-sampler is designed for water sampling and is built based on the BoSL FAL Pump (McCarthy *et al.*, 2021).

The BoSL FAL Pump is a 3D-printed and cheap peristaltic pump, and it is operated on Arduino (an open-source electronic prototype platform) using widely available hardware components. The BoSL FAL Pump consists of a 5-12v direct current motor, 3D-printed rotating wheel and cases, and silicon tubing. The pump can be powered by low-voltage rechargeable batteries and operated by an Arduino microcontroller board and a MOSFET transistor. A magnet (installed on the rotating wheel) and a hall effect sensor (connected with Arduino board to detect the magnetic field change) are added as an option to count the number of the pump rotations. The operation of the BoSL FAL Pump can be flexibly programmed to meet the sampling requirement. Users can define three variables: (1) number of minutes between each pumping operation, (2) number of rotations in one pumping operation, (3) duration of the run (McCarthy *et al.*, 2021). However, a complete hardware design is still needed to make the BoSL FAL Pump practically used in water sampling.

The MAD-AS auto-sampler uses 3D-printed outer cases and centrifuge tubes to provide waterproof housing to the BoSL FAL Pump. A 3D-printed container is designed to be placed in the centrifuge tubes and hold the batteries, circuit, microcontroller, and pump securely. The microcontroller used in MAD-AS is the specifically optimised MicroBoSL (Catsamas, 2021), a miniaturised board. Normal Arduino boards are not recommended as it is too big to fit in the centrifuge tubes. An upwards-curved 3D-printed front is installed at the upstream end of the MAD-AS. Small holes on this component allow the water to enter its chamber and be collected through the silicon tube (pump inlet). These holes prevent large debris from contacting the pump inlet directly (Figure 1.1). A small 3D-printed filtering component is installed at the silicon pipe inlet to provide further protection from blockage and clogging (Figure 1.2). The pump outlet is connected to a polyvinyl chloride pipe (PVC pipe, 19mm in diameter) installed at the top of this upwards-curved front. The PVC pipe provides storage space for the collected water sample. A 1-

Table 1. Design File of MAD-AS auto-sampler and BoSL FAL Pump

Design File Name	***File Type***	***Opensource License***	***File Location***
BoSL_FAL_Pump.stl	*STL*	*CC BY 4.0*	*http://dx.doi.org/10.17632/prb2wzr77y.1*
BoSL_FAL_Pump_1.ino	*Arduino IDE code*	*CC BY 4.0*	
BoSL_MAD_AS_Container.stl	*STL*	*N/A*	*https://drive.google.com/drive/folders/1BX3I7SSH7kCZtCH1Oy_nSIFs5DQmqzZW?usp=sharing*
InsewerPumpCase.stl	*STL*	*N/A*	

meter-long PVC pipe can store approximately 283 millilitres of sample.

2.2 *Design File*

The design files of MAD-AS and BoSL FAL Pump are listed in Table 1. The Arduino code is written by McCarthy *et al.* (2021) and loaded into the MicroBoSL board to control the pumping operation. The STL (Standard Tessellation Language) files are used to 3D-print the components (pump, container, and outer case).

2.3 *Bills of Material*

Table 2 lists the components and costs of the MAD-AS Auto-Sampler. The total cost of an auto-sampler is around 70 Australian dollars, given that the listed components are high-quality products, and the listed prices are the retailing prices in Australia. The unit cost of MAD-AS can be halved if supplied from overseas wholesalers.

2.4 *Hardware Building Instruction*

McCarthy *et al.* (2021) provided instructions on the 3D printing set-up and rotating wheels and pump assembly. Before assembling the rotating wheels, all the 3D-printed components that contact directly with the silicon tube when the pump is operating should be treated using sandpaper. If the component surface is not smooth and has excess printing material, the silicon pipe would be abraded and broken quickly, and water leakage will destroy all electronic components.

Table 2. Bills of Material of MAD-AS Auto-Sampler

Component	***Number***	***Cost Per Unit [AUD]***	***Total Cost [AUD]***
1.75mm PLA Filament for 3D printing	*156g*	*$0.026*	*$4.06*
MicroBoSL Board	*1*	*$15*	*$15*
3 pins SPDT Slide Switch, COM-09609	*1*	*$1.17*	*$1.17*
Rechargeable Battery, RCR123A	*2*	*$11.50*	*$23*
Hall Effect Sensor, A1309KUA-9-T	*1*	*$2.80*	*$2.80*
Neodymium Magnet, M1219-2	*1*	*$1.34*	*$1.34*
75 RPM, 6V, DC Motor, COM0806	*1*	*$7.38*	*$7.38*
1.8mm diameter, 13mm high Pins for rotating wheel	*3*	*$0.13*	*$0.39*
Networking Cable	*0.3m*	*$0.0492*	*$0.02*
2mm ID, 4mm OD, Silicon Tubing	*100mm*	*$0.0037*	*$0.37*
50mL, 28mm ID, Centrifuge Tubes	*2*	*$0.72*	*$1.44*
Cable Gland, M8	*5*	*$0.7*	*$3.5*
22mm ID, 27mm OD, Rubber O-rings	*4*	*$0.654*	*$2.62*
19mm ID, PVC Tubing	*1m*	*$7.12*	*$7.12*

The wiring of the control system is altered because the auto-sampler uses a MicroBoSL board (see Figure 2). The MOSFET transistor is pre-installed on the MicroBoSL board. Hence no external MOSFET is needed (to control the power of the pump). Hot glue can be applied as electrical insulation to avoid short circuit that may happen when the control system vibrates in the narrow and small housing with the pump.

After assembling the electrical circuit in the 3D-printed container and placing the pump above the hall effect sensor using hot glue, the core part of the MAD-AS auto-sampler needs to be installed in the centrifuge tubes and then outer case carefully to ensure waterproofness.

Two centrifuge tubes (50mL volume, 28mm internal diameter), one of which needs to be cut short, are plugged together to house the control system container. Two holes are drilled on the tube cap to allow the silicon pipe to connect with the pump inside the centrifuge tubes through cable glands (see Figure 2.7). The cable glands should be installed and fastened tightly. Grease (such as petroleum jelly) should be applied to every joint, that is, the joint of the pump housing and the centrifuge tube, and the O-rings inside the cable glands. The purpose of grease is to make assembly easier and, more importantly, to add extra protection from water penetration. However, the joint between the two centrifuge tubes is an important exception because it is easy for the centrifuge tubes, with grease lubricating at the joint, to disconnect when the pump is operating and vibrating. After connecting the centrifuge tubes, a visual inspection is needed to check if they are jointed in a straight line (that is if their entire interfaces are fitted tightly) to avoid water penetration due to the loose joint.

The 3D-printed outer case provides another waterproof protection, mitigates clogging caused by wastewater debris, and facilitates the connection between the pump and the sample storage space. Similar to the design idea of using two centrifuge tubes, the outer case consists of two sections and a cap (see Figure 1.3). Two holes on the case cap are also used for the in and out of the silicon tube. In the design, these two holes do not have any thread, and it is not recommended to use the thread drill, which may make the installation of the cable glands easier but also allow space for water penetration. The cable glands should be carefully and forcefully screwed into the holes vertically using a plier. After the cable glands are screwed tightly into the cap holes, hot glue should be applied to seal around the cable glands at the back of the cap. Two O-rings fittings are designed at each joint (on the cap and the lower section, for them to connect with the upper section), instead of using screws for connection. The O-rings can tighten the case joints and avoid water leakage. These O-rings should be lubricated thoroughly using grease. Otherwise, they can be easily broken when the case component is pushed hard into the other. Grease should also be applied around the joint interfaces. Hammer should not be used to help to push the case components, because it can easily damage the case structure and break the O-rings.

The last step is to install the case front and the PVC tubing. The case front is connected to the main body using a screw. The pump inlet remains inside the chamber of the case front, where it pumps the water sample. The pump outlet is longer and extended to the sample storage space at the top end of the case front through the cable gland. The storage tubing (PCV pipe) has a diameter smaller than the diameter of the top of the case front. Hence it should be heated and softened by a heat gun before being pushed onto the top of the front case. After the pipe cools down, it shrinks and wraps tightly and prevents the collected sample from leaking back to the environment. After installation, wrap all joints

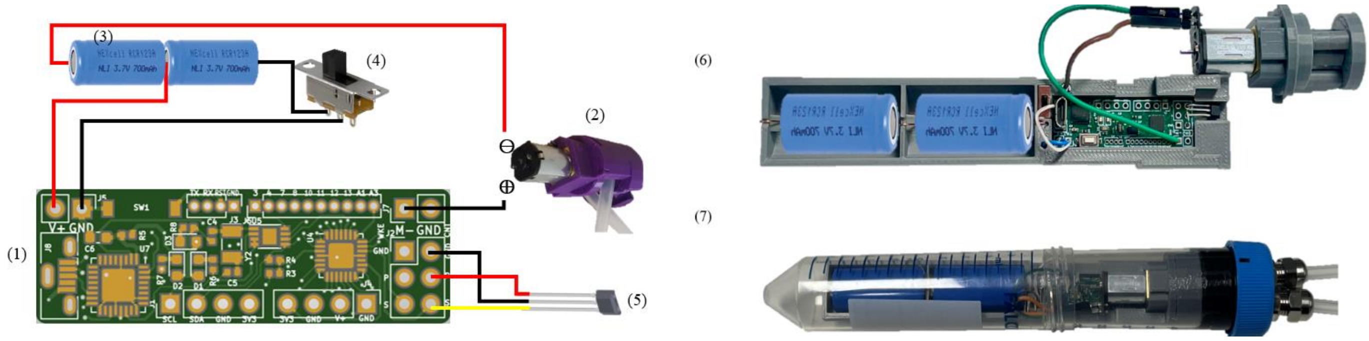

Figure 2. Left: Wiring Diagram using a MicroBoSL board (not to scale). Right: Assembly of the control system and centrifuge tubes. (1) MicroBoSL board with built-in MOSFET. (2) BoSL FAL Pump: The motor polarity is switched because the pump is designed to operate in the clockwise direction. (3) 3.7V Lithium-ion Rechargeable Batteries: MicroBoSL board needs only one battery to operate, but two batteries are needed to power the motor. (4) Slide Switch: To turn on/off the control system. (5) Hall Effect Sensor: Pins are directly soldered on the board without wire. (6) Core Component: The control system case is designed to house the batteries, switch, microcontroller, and the BoSL FAL Pump securely. (7) Assembled control system in the centrifuge tubes.

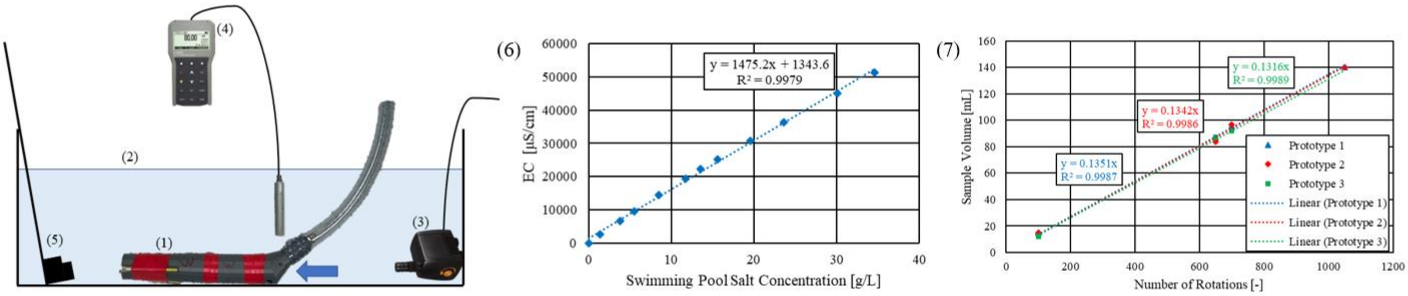

Figure 3. Experiment Set-up and Experiment Linear Regressions. (1) MAD-AS Auto-Sampler. (2) Deionised Water. (3) Pond Pump. (4) Portable EC Meter. (5) BoSL All-In-One Senor. (6) Linear Regression: EC versus Salt Concertation. (7) Linear Regression: Sampled Volume versus Number of Pump Rotations.

with electrical tape to secure the connections and prevent water leakage.

2.5 *Hardware Operation Instruction*

The Arduino code file can be edited using Arduino IDE on a computer. The operation set-up (number of minutes between each pumping operation, number of rotations in one pumping operation, and duration of run) needs to be defined before being uploaded to the MicroBoSL board through the micro-USB port on the board. Turn on the switch and the pump are ready to be deployed.

The entire MAD-AS Auto-Sampler can be submerged underwater if the top of the PVC tube is sealed securely. Otherwise, the top of the PVC pipe should remain above the water level, and it is recommended to be attached to any object above the water level using cable ties.

2.6 *Constant Increments Validation*

Three MAD-AS Auto-Sampler prototypes have been built to triplicate the laboratory experiment and mitigate the potential errors and gain more presentative results.

McCarthy *et al.* (2021) validated that, in intermittent pulse-based pumping operation, the flow rate of the pump was constant in 0.14mL volume increments (0.14mL of water could be pumped for every rotation). Nevertheless, an alternative microcontroller and different battery voltage (two 3.7V batteries) had been used in the MAD-AS Auto-Sampler. It was necessary to test if constant volume increment was still validated for the modified design. Also, a constant volume increment within a sampling deployment is important for time-based sampling. To validate it, the prototypes were set up with different numbers of minutes between each pumping operation or numbers of rotations in one pumping operation, and the prototypes were tested on different days and different total durations. The volumes of collected water sample were measured using a measuring cylinder and recorded with the total number of pumping rotations taken to collect the sample.

2.7 *Laboratory Test Setup*

In the preliminary laboratory test, the “virus in wastewater” event was simulated in a large bucket, using deionised water (DI water) and saltwater (EC solution). The electrical conductivity (EC) of water in the bucket was the simulation of the virus concentration in the wastewater. The electrical conductivity could be changed by adding DI water or EC solution, and it could be easily measured. Three experiments were performed to simulate three “virus in wastewater” scenarios. (1) Slow increase and slow decrease in concentration, (2) rapid peak and rapid decrease in concentration, and (3) rapid peak and slow decrease in concentration. This preliminary test aimed to examine that if and how the water sample collected by the prototypes were able to represent the EC over the operation period.

The experiment was set up as demonstrated in Figure 3. The DI water with zero EC was added before the experiment started. A pond pump (AP550, Aquapro, Australia) was installed with the pump outlet placed in the horizontal direction, it would be used to circulate water in the bucket and speed up the mixing of the added solution. Two BoSL All-In-One Sensors (Shi *et al.*, 2021) were installed to record the electrical conductivity every one minute in the data logger. A portable EC meter (HI98192, Hanna, USA) was also used to manually record the electrical conductivity as a backup, each time after the solution was added into the bucket. The prototypes were submerged into the water (extra weight may be required to prevent them from floating). The PVC pipes were fixed to nearby table legs using cable ties.

The saltwater (EC solution) was prepared using DI water and swimming pool salt (Sunray, Australia). Before the experiment, the linear relationship between salt concentration (weight of salt per unit volume of water [g/L]) and the electrical conductivity was derived by adding salt into the testing solution and recording EC values (see Figure 3.6). Therefore, we could calculate the amount of salt or water needed to change the EC in the bucket to the target level.

During the experiment, adding EC solution increased EC value and adding DI water reduced EC value. Each time before the EC solution was added into the bucket, a certain amount of salt was added and mixed with 500mL of DI water using the magnetic stirrer to make sure the salt could be fully dissolved quickly. Then, added EC solution or DI water could be circulated and mixed with the existing water in the bucket evenly and quickly by the pond pump. Each time the EC level was changed, the time, the weight of added salt, the volume of added DI water and EC meter readings were recorded for later calculation. Six EC meter readings were taken in different positions in the bucket to get an average value and to check if the EC solution or DI water is evenly distributed.

The composite sample collected by the MAD-AS Auto-Sampler was expected to have a time-weighted average salt concentration or EC value. "Time-weighted average" means that a constant volume of sample is collected in each sampling operation. If the pump operated as we expected, the concentration or the EC value of the collected sample could be calculated using the time-weighted average equation (see Equation 1):

$$\overline{EC} = \frac{\sum_{i=1}^{n}(EC_i \cdot t_i)}{\sum_{i=1}^{n} t_i} \quad (1)$$

where $\overline{EC}$ = calculated EC value of the composite sample, EC_i = EC value in the bucket when the ith sampling operation was performed, t_i = the time interval between the ith sampling operation and the next operation. In this experiment, the sampling time interval was also fixed, which was 1 minute. Hence, the equation could be simplified into Equation 2:

$$\overline{EC} = \frac{\sum_{i=1}^{n}(EC_i)}{T} \quad (2)$$

where T = total duration of deployment (experiment). Then, the actual EC value of the collected sample should be compared with the calculated EC value.

2.8 *Wastewater Treatment Plant Trial Deployment*

Two MAD-AS Auto-Sampler prototypes and one torpedo-style passive sampler (Schang *et al.*, 2020) were deployed to Aurora Wastewater Treatment Plant for one day after the SARS-CoV-2 fragments were detected there in late April 2021. The first MAD-AS and the passive sampler were deployed for one day from 29th April 2021, and the other MAD-AS was deployed for three days from 30th April 2021. The samples were transported to the laboratory on ice. Pre-processing, RNA extraction, reverse transcription and qPCR tested were performed in accordance with Schang *et al.* (2020).

3 RESULTS

3.1 *Constant Increments Validation*

Linear regression analysis was conducted, and it was found that all three MAD-AS prototypes had a linear relationship between the number of rotations and the sampled volume (all R^2 values were larger than 0.99, see Figure 3.7). On average, more than 0.13 millilitres of water could be sampled in one rotation. The values were slightly lower than the 0.14 mL in McCarthy *et al.* (2021). The possible reasons were the sample loss and human error during measurement, so the constant volume increment could still be validated. This validation proves that an assumption of the time-weighted average approach is not violated (volume increment in each pumping operation is constant during the entire run).

3.2 *Laboratory Test*

All the lab experiments ran over 1 hour. The sample volumes collected in test 1 were approximately 140mL and 140mL for prototype 1 and 2. The sample volumes collected in test 2 were 87mL, 84mL and 87mL for prototype 1, 2 and 3. The sample volumes collected in test 3 were 96mL, 97mL and 92mL for prototype 1, 2 and 3. All prototypes can collect approximately the same volumes of samples in each experiment.

The background EC changes in the experiments and the comparison between expected composite EC and actual sample ECs are plotted in Figure 4. The absolute differences between predicted and actual EC in scenario 1 were -0.18mS/cm and 0.16mS/cm (% error: -0.99% and 0.90%) for prototype 1 and 2, respectively. The absolute differences between predicted and actual EC in scenario 2 were -0.20mS/cm, 0.14mS/cm and -0.37mS/cm (% error: 5.9%, 4.2% and -11%) for prototype 1, 2 and 3, respectively. The absolute differences between predicted and actual EC in scenario 3 were -0.17mS/cm, -0.13mS/cm and -1.1mS/cm (% error: -3.1%, -2.4% and -20%) for prototype 1, 2 and 3, respectively. The results of MAD-AS prototype 1 and 2 were accurate and stable over the three individual tests. The absolute differences were marginal and never exceeded 0.2mS/cm. The percentage errors in the latter two tests were higher, but it was because the background EC used in test 1 was higher. The results from MAD-AS prototype 3 were less promising, because its absolute differences were higher, up to 1.06mS/cm, which was almost a 20% error on average across the simulated event. Nevertheless, a

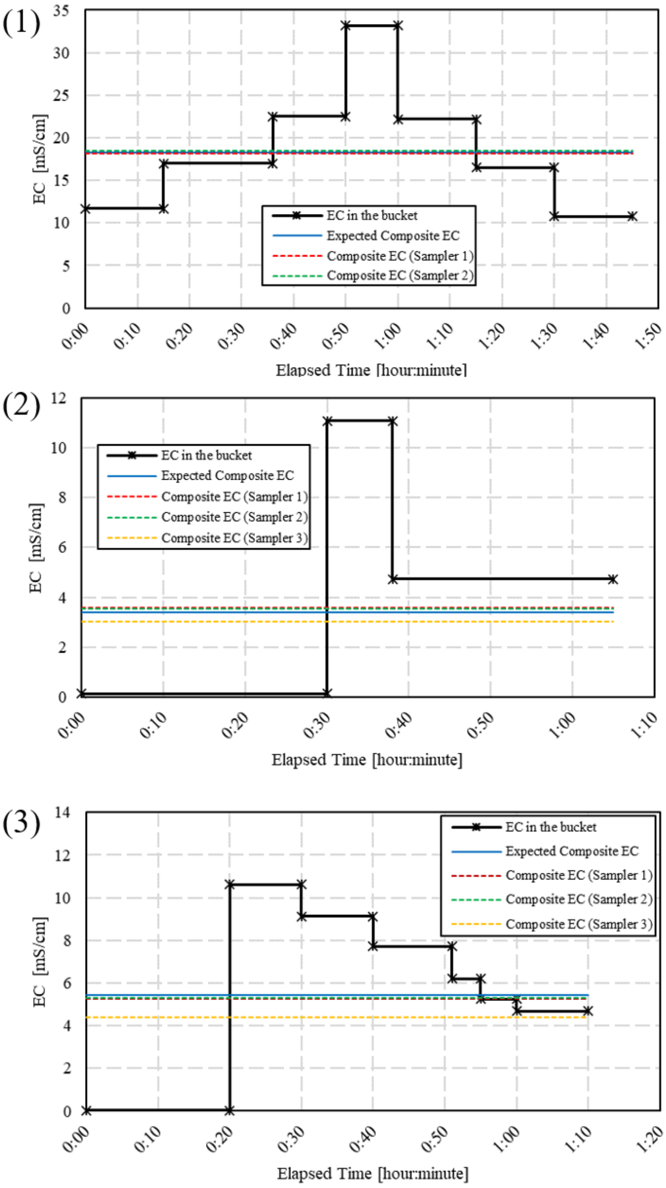

Figure 4. Experiment Results. (1) slow increase and slow decrease. (2) rapid peak and rapid decrease. (3) rapid peak and slow decrease.

20% uncertainty is not considered as a large uncertainty from the microbe perspective, because the error in the assay of an E. Coli sample can be greater than 30% (McCarthy *et al.*, 2008).

No water leakage into the sampler case and control system was observed in all experiments.

3.3 *Trial Deployment*

Around 250mL and 750mL sample were collected in 1-day sampling and 3-day sampling respectively (sampling interval was set to 6 minutes) (see Figure 5). No clogging or blockage, or water leakage was observed on the MAD-AS samplers. However, both MAD-AS and passive samples were tested negative for SARS-CoV-2.

4 DISCUSSIONS

4.1 *Performance*

The MAD-AS design is capable to automatically and frequently collect and store composite water sample with good temporal resolution and it meets all the desired features which it was wanted to have. The components of a MAD-AS cost only 70 Australian Dollars which can be even cheaper if bought from the wholesalers. The sampler is small and can be deployed easily in the wastewater treatment plant or sewer network through a small access hole, minimising the OHS issue to the workers. The workers only need to be present when deploying and retrieving the sampler. The sampler does not require external power access, the current design configuration allows the sampler to operate for more than 7 days, with two 3.7V 700mAh batteries. Also, the sampler can be reused after replacing the rechargeable batteries and the silicon tubing (which may not be durable to be used multiple times). If it is used to sample wastewater, the sampler can be disinfected safely by bleach without being harmed or corroded. Also, the design of the case front and filtering component significantly mitigate the clogging and blockage problems that happened to the passive samplers (Schang *et al.*, 2020).

Although the other two prototypes can preliminarily prove that this innovative and

Figure 5. Trial Deployment. (1) Both MAD-AS and the passive sampler were deployed at the same location at the treatment plant inlet. (2) Samplers submerging in the wastewater flow. (3) Sample collected by MAD-AS. (4) The passive sample under pre-processing in the laboratory.

inexpensive auto-sampler can be used for time-based automatic sampling, the issues that caused the inaccuracy of prototype 3 should be investigated. One of the most possible problems is that the pump in prototype 3 stopped working during random periods in the experiments but re-started again after a random time. In the Arduino program, the pump needed to re-calibrate itself by performing rotations, which are more than the number of rotations during a normal pumping operation. By this, the re-calibration still "helped" to collect the same total volume of the sample as the others, but the volume increments over the entire test were not constant, making the actual composite EC differ from the estimated EC (which was calculated, assuming that constant volumes could be collected each minute). The reasons that may cause the stopping and restart of the Arduino board were likely to be the dip in power. However, these are the assumptions only, more tests on MAD-AS are required to validate the reliability of the design and find out the issues.

4.2 *Limitations*

This MAD-AS auto-sampler has got the features needed to be an alternative sampling technique and remedy the shortcomings of other sampling techniques. However, due to the time constraint of this study, only one trial field deployment was conducted. The Aurora Wastewater Treatment Plant deployment did not detect the SARS-CoV-2 fragments because it is likely that there was no SARS-CoV-2, in fact. Therefore, the capability and efficiency of MAD-AS in detecting SARS-CoV-2 in wastewater have not been validated yet. In addition, after validating the efficiency, further study is still needed to examine how MAD-AS helps obtain quantifiable results – the quantitative analysis to predict the number of infected people in the catchment.

If MAD-AS is used in natural waterway sampling, a limitation would be that the collected sample becomes less representative after the storm/high flow event. MAD-AS is a time-based sampler, it collects a constant volume of the sample after a pre-defined time interval. When there is fluctuation in the flow rate, MAD-AS is not able to vary the volume that is proportional to the flow rate. When high flow events happen, the events' significant effect (for example, rainfall brings large pollutant load to the river) on water quality and characteristics will be overlooked by MAD-AS. This issue can only be solved if MAD-AS can measure the flow rate of the stream and vary the sampling volume every time. This flow-based sampling function is mostly seen on the comprehensive automated sampling machines.

It should be noticed that electrical conductivity is the parameter that measures salts or other chemicals dissolved in the water. Since EC is highly dissolved and highly universally distributed, the uncertainty of the experiment is isolated. The sampler can have better performance and obtain relatively accurate results about the EC. But it remains unknown how well MAD-AS can capture the other parameters on the undissolved nutrient and contaminant. For example, the suspended solids are a kind of undissolved pollutant. They are less evenly distributed in the waterways, and the particles can settle in the water due to gravity. The inherent uncertainty will adversely affect the performance of the MAD-AS. Although it is not the problem of MAD-AS, how accurately it can obtain results about the other parameters should be under further investigation.

4.3 *Future Work*

The experiment using EC solution was a preliminary test in a controlled laboratory environment. Further experiments in the field are required to validate the feasibility and reliability of the MAD-AS in different environment and location scenarios. To validate the application in wastewater surveillance, MAD-AS auto-samplers should be deployed to various kinds of locations (treatment plant, manhole, and sewer pipeline), and compare with other commonly used sampling methods to examine if and how well MAD-AS auto-samplers could capture the viruses, such as SARS-CoV-2 and E. coli. To validate the application in natural waterways, MAD-AS auto-samplers can be deployed to locations close to hydrological stations. Water parameters can be compared between the MAD-AS sample and the data recorded by the hydrological stations.

5 CONCLUSION

An inexpensive and innovative sampling method for wastewater-based epidemiology (such as SARS-CoV-2 near-source tracking) has been designed and preliminarily evaluated. The new sampling method aims to provide an alternative wastewater sampling approach free of many limitations the other commonly used sampling methods have. The waterproof MAD-AS auto-sampler is built using 3D-printed components and the Arduino-operated control system, which are all widely available, low-cost, and reusable. It can automatically collect the water sample in good temporal resolution. The MAD-AS is also designed to minimise the workers' exposure to wastewater and mitigate the clogging and blockage caused by the debris in the wastewater.

The auto-sampler prototypes effectively collect composite EC solution sample in the controlled laboratory environment, that is representative of the variation in the environment EC (electrical conductivity) over a period of time. The absolute differences between the actual composite EC value and the expected EC value (time-weighted average) are smaller than 0.2mS/cm in most of the cases. A trial deployment to wastewater treatment plant also proves that the auto-samplers can work as designed and are waterproof and clogging-free. Since the conceptual design of this auto-sampler is validated, further field experiments have been recommended to examine the feasibility and accuracy in different scenarios, which includes deployment to the wastewater treatment facility and pipeline network, and also deployment to the natural waterways as the sampling method can be potentially extended to the use in stormwater and natural water sampling.

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Management Statement

I am satisfied with the outcomes I have got for this final year project, given that I must complete the laboratory-based project in 12-week time. I am only eligible to take a 6-credit-point project due to the course structure requirement, but I still chose the lab-based project because I was keen to be involved in this project. I expected that this would be challenging, so I tried to get myself prepared for this project.

However, I think I did not do well in time management because I could not arrange enough time for the early laboratory work, which was mainly the assembly of the MAD-AS prototypes. I underestimated the difficulties in assembling the auto-samplers from an untrained undergraduate student's perspective. In addition, as this MAD-AS project has not been done by anyone and my supervisor started the project not much earlier than me, I had to solve many first-hand problems on the assembly with my supervisor. It was a rewarding process but also took a lot of my time. Therefore, the delays caused by different issues made me change my proposed schedules, and I only had one chance to deploy the prototype to the wastewater treatment plant. And the only deployment was in a bit of bad luck because both MAD-AS and another sampling method did not detect the novel coronavirus, SARS-CoV-2. But the negative result was expected because in fact, Melbourne did not have any COVID-19 outbreak, and it was challenging to find the virus fragments in the wastewater. If time was sufficient, more deployments to the treatment plant or other parts of the sewer system might give us more chances to find the virus (as Melbourne still has a few COVID cases in the hotel quarantine, or the recovered patients may shed the virus out of their bodies). Once positive detection is found, the data can make the validation much more robust and turn our assumption into a more solid conclusion. Therefore, back to the issue of time management, the impact of uncertainties and delays are sequential. The problems that happened in the beginning, would have a significant impact on the outcomes. I should have expected the uncertainties of the project and manage my time well and give allowance to any delay.

Positive detection in more deployments is optimistic anticipation. I am still happy with the actual results I got in this paper because I also set up a laboratory experiment to evaluate the performance of the prototypes. My supervisor provided me with several good ideas on how to set up the experiment as an alternative way to validate the design. Then I designed and set up the experiment on my own. The laboratory experiment looked very easy. It was simple only in the theory we used, but there was way more work needed to get the simple experiment done. For example, I needed to prepare an experiment plan to guide me through the experiment without hurry and confusion. Then, I prepared a spreadsheet to calculate the salt and water that I needed to add to each step of the experiment plan. Therefore, I learnt the importance of preparing a detailed plan for the experiment, and we should never assume the experiment can be done quickly without any written procedure documents.

In the entire laboratory experiment, I learnt and used different toolboxes more than I thought. Hence, sometimes I used a lot of time on a simple task because I was unfamiliar with the tools and had difficulties using them. Though I gradually got familiar with using these tools, it should have been better to know how to use them before the lab work as most tools are commonly used in our lives. The essential tool skills can help to solve many issues in lab-based academic work.

The management of the literature review was more difficult than I expected. I changed and learnt several reference management software to find the most suitable one. I found it was crucial to note down the key points and highlights of every piece of literature I had read in one document. Otherwise, the more paper I read, the more quickly I could forget. The notes helped me retrieve the key information of the paper I had read. This significantly saved my time as I quickly found the most suitable reference for a statement in my FYP paper without the need to open and re-read the literature.

Other than the academic perspective of this project, I also learnt a lot of other skills which are important for my future career, no matter in research field or in business workplace. I learnt communication skills because I had to communicate frequently with my supervisor and co-supervisor about the design and assembly. I boosted my attention to details and self-learning ability as well.