Comparison of pathogen die-off patterns of tomatoes grown in two hydroponics systems

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Abstract

Due to water shortages in most parts of the world, alternative water sources are required for daily activities, such as agriculture and domestic uses. Treated domestic wastewater reuse is gaining acceptance around the world, mainly for non-human contact use. Research is being conducted in using treated domestic effluent to grow edible food crops. However, one of the major concerns with wastewater reuse for food production is the risk of pathogen contamination to the edible parts of the food and to the people exposed to irrigation.

Wastewater application in horticulture using hydroponics technology should minimise the exposure and contamination risk to the workers. Since the edible parts of the plant, with the exception of root crops, may not be in direct contact with the wastewater, contamination to the edible parts may also be reduced. This chapter examined two hydroponics systems, nutrient film technique and water culture (without aeration), for their efficiency in causing pathogen die-off. Three treatments, secondary treated domestic wastewater, control medium (commercial hydroponics medium) and pathogen spiked control medium were tested in triplicate. S.typhimurium (ATCCI4028) and E.coli (WACC4) were used to spike one of the treatments (spiked control medium). The experiment was conducted over four months with the medium changed every fortnight. The results showed that there was a general decrease of pathogens over seven days (>40%) in the medium and complete die-off was observed after 14 days (99%), in both types of hydroponics systems. In both systems, there were no pathogens detected in the fruits. The hydroponics techniques for domestic effluent reuse, is a viable option for edible crop production as it reduces the risks of bacterial pathogen contamination.
Introduction

The risk of pathogen contamination of edible food crops has limited the use of treated wastewater for crop production. The risk to humans through contact with wastewater reuse, involves transmission of pathogens including infectious enteroviruses (Mignotte et al., 1999), especially in edible food crops. When growing crops in effluent, it is necessary to consider the risks involved, especially if the crops are for sale or consumption (Ottoson et al., 2005). Amahmid et al. (1999) found *Giardia* cysts and *Ascaris* eggs on crops irrigated with raw wastewater, however, not on crops irrigated with treated wastewater.

Different types of irrigation systems can be used in reducing the risk of transferring contaminants to plants and workers. Irrigation systems such as sprinkler and open irrigation, where humans are in contact with the effluent are not widely accepted due to the associated health risks. There may be risks involved with using effluent for soil irrigation as it may contaminate edible food crops through direct contact with the plants (Rosas et al., 1984). Soil and groundwater contamination with pathogens and parasites is also possible through soil irrigation.

The nutrient film technique (NFT) and water culture (WC) are forms of hydroponics that may reduce these risks compared to other irrigation systems. If the hydroponics technique is used for growing leafy and fruit crops the edible parts of the plant are not in contact with the wastewater because a physical barrier is placed between the plant parts and medium. The other advantage of this system is that it is a type of intensive agriculture where farmers/communities are able to grow substantial amount of crops in limited space.

This chapter looked at the possibility of bacterial contamination of plants if wastewater was used to grow edible crops in hydroponics systems using the NFT and WC system. The suitability of tomatoes grown in secondary treated effluent for human consumption was determined. It also examined the bacterial pathogen die off rate in the solution at different nutrient solution retention times. Although parasites are the main concern in wastewater irrigation in most developing countries, their level in secondary treated effluent from Australia are negligible therefore only bacterial pathogens were tested.

Materials and Methods

The nutrient film technique (NFT) experiment was set-up as shown in figure 1 and the water culture (WC) experiment was set-up as shown in figure 2.

Wastewater and Control Medium

The secondary treated domestic wastewater was collected from a domestic wastewater treatment plant (Perth, Western Australia) in 200L drums for the experiments. The control medium used was a
commercially available hydroponics nutrient solution (Ag-grow) for fruits and vegetables. This nutrient solution was chosen for this study as it was readily available and it was the most popular in the store. The control treatment was prepared as per the specified ratio of SmL of hydroponics medium to 1L of water, as recommended by the manufacturer.

Experiment Design

The experiment was conducted in a greenhouse to provide uniform conditions throughout the growth phase. In the NFT experiment, secondary treated wastewater was pumped from a 42L reservoir to the channel where plants were grown. The 295cm x 12cm x 12cm channels had an inlet and outlet leading to the reservoir. The volume of solution in channels at any one time was approximately 7L. The effluent was drained by gravity flow back into the reservoir. Each tray was set up as shown in figure 1. Uniform-sized plant seedlings were purchased from a commercial nursery and planted in a pot containing expanded clay balls, which were used as the bedding material and inserted into the eight planting slots of each channel. Pumping from the reservoir into the channels and recirculation of effluent was considered to provide adequate aeration.

In the WC system, the plants were grown in tubs containing the nutrient medium. It was a closed-system without recirculation of the nutrient medium. The WC nutrient solution retention time was 14 days. The tomato seedlings were planted in 10cm x 10cm nursery pots, filled with expanded clay balls and then suspended into the tubs to allow the roots to grow into the nutrient solution. Both experiments were conducted in triplicate.

Figure 1. NFT experiment design
The commercial nutrient solutions were inoculated with *S.* *typhimurium* and *E.* *coli*. Pure cultures of *Salmonella typhimurium* (ATCC14028) and *Escherichia coli* (WACC4) used for this experiment were grown in buffered peptone water and lauryl tryptose broth respectively. Serial dilutions were prepared according to Standards Australia (1991), method DR88082. The pathogens from the pure culture were pipetted (1mL) into Mcartney bottles containing 9mL sterile water, and was then spiked to the treatment (storage containers). Sampling of wastewater (WW), control medium (CM) and spiked control medium (CMS) was conducted every 7 days and after every 14 days when the medium was changed and pathogens were inoculated. The experiment was conducted for 35 days and was spiked three times.

For analysis, the water samples were collected in sterile 250mL schott bottles and tested following the methods by Standards Australia for both *E.* *coli* (Standards Australia., 1995a), method DR93215 and *S.* *typhimurium* (Standards Australia., 1995b), method DR93222.

When the plants were ready for harvest, the edible parts of the plants were separated and were tested. Organically grown tomatoes were purchased from a supermarket for comparison of the quality. The edible parts of the plants were washed with sterile water and the wash water was analysed for *E.* *coli* (Standards Australia., 1992), method DR91155 and *S.* *typhimurium* (Standards Australia., 2004), method DR07430CP.

To determine whether there was a significant difference between the media samples and the plant samples, results of the experiments were analysed using Independent-Samples T Test and One-Way ANOVA.
Results

Overall, NFT had a better pathogen reduction rate than the WC within the first seven days (table 1). There were no detectable pathogens after 14 days in both systems (table 2).

Table 1. Percentage reduction of bacterial pathogens in NFT and WC systems in 7 days

<table>
<thead>
<tr>
<th>Spike</th>
<th>Percentage reduction in pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NFT (7 days)</td>
</tr>
<tr>
<td></td>
<td>WW</td>
</tr>
<tr>
<td>1st spike</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>100</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>100</td>
</tr>
<tr>
<td>2nd spike</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>100</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>100</td>
</tr>
<tr>
<td>3rd spike</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>88</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>67</td>
</tr>
</tbody>
</table>

n/a – not applicable

Table 2. Percentage reduction of bacterial pathogens in NFT and WC systems between 7 and 14 days

<table>
<thead>
<tr>
<th>Spike</th>
<th>Pathogen</th>
<th>Percentage reduction in pathogens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NFT (14 days)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WW</td>
</tr>
<tr>
<td>1st spike</td>
<td></td>
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</tr>
<tr>
<td>E. coli</td>
<td>100</td>
<td>100</td>
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<tr>
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<td>2nd spike</td>
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</tr>
<tr>
<td>E. coli</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Harvest

Pathogen Concentration in Wash Water

The wash water from WW, CM, CMS and organically grown tomatoes had no pathogens detected (table 3).

Table 3. Number of bacterial pathogen contamination in silver beet and tomato wash water (100mL) in NFT and we (WW, eM, eMS), organically grown (O)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>NFT</th>
<th>WC</th>
<th>Supermarket</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WW</td>
<td>CM</td>
<td>CMS</td>
</tr>
</tbody>
</table>

n.d. – not detected

Pathogen Concentration on the Edible Parts of the Plants

There were no detectable concentrations of E. coli or S. typhimurium in any of the edible parts of the plants grown in WW, CM, CMS and 0 tomatoes.

Discussion

The die-off rate was quite significant in most cases (tables 1 and 2). Pathogens were significantly reduced in most samples by the 7th day, which is less time (between 14 - 21 days) than was noted by Oyama et al. (2008). The die off rate was higher in the NFT than WC, this may be due to the aeration in the NFT system. The microbial effluent quality after 14 days was within the World Health Organisation guidelines (WHO, 1989). The revised guidelines state that drip irrigation with treated wastewater should contain less than 1000cfu/100mL E. coli for low growing crops and less than 100000cfu/100mL E. coli for higher growing crops (WHO, 2006). The Australian (ARMC (Australia) et al., 2000) guidelines for raw edible food crops in contact with treated effluent should have thermotolerant coliform count of less than 10cfu/100ml and crops not in direct contact with treated effluent should have a thermotolerant coliform count of less than 1000cfu/100ml. The effluent quality of the medium was well below these guidelines. The validity of using faecal coliforms as an indicator of pathogens has been questioned, however, Harwood et al. (2005) found that it was an adequate indicator to use in order to protect human health.

A study conducted by Teltsch and Katzenelson (1978) showed a strong possibility that enteric bacteria and viruses can be spread in the air via spray irrigation. Studies have shown the possibility of contaminated water used for spray irrigation may play a big role in contaminating vegetables (Islam et
It was observed that crops like lettuce and parsley irrigated with raw wastewater were more contaminated compared to crops like tomatoes and pimento (Melloul et al., 2001). The most likely reason given was that leafy vegetables that develop at the soil surface have more foliage, which offers more area for contamination from water spray (Melloul et al., 2001; Rosas et al., 1984). Samples of lettuce and radish grown in soil fertilised with manure and fertiliser are observed to be contaminated with faecal coliforms which could have been due to contact of the vegetables with soil (Machado et al., 2006).

Using alternative methods for wastewater irrigation can reduce the exposure of edible parts to pathogens (NRMMC et al., 2006). This study revealed that growing vegetables using the nutrient film technique in hydroponics system could reduce the exposure risk. However, a study showed that hydroponic tomatoes grown in an inoculated nutrient solution was found to take up S. typhimurium to the stems and leaves of young plants (before fruit maturation) (Guo et al., 2002). In this study (table 3), tomatoes grown in WW and CMS were not contaminated with the pathogen. One reason may be because there is no contact between the edible parts of the plant and the contaminated medium.

Tomato fruits have the ability to promote salmonella growth, depending on the handling methods (Zhuang et al., 1995). Abdul-Raouf et al. (1993) found that it was possible to contaminate salad vegetables with E. coli 0157:H7 during production, harvest, processing, marketing and preparation. As a result care should be taken when handling the vegetables. Another possible source of contamination of food crops is through the water sprinkled on vegetables in order for them to look fresh (Hamilton et al., 2006) for marketing purposes.

As bacterial survival depends on climatic conditions, the results may vary from region to region (Vaz da Costa-Vargas et al., 1991). In this study, pathogen contamination of vegetables using secondary treated domestic wastewater is significantly low and as a result can be recommended for safe consumption. The effluent after passing through the hydroponics system, for a period of 7-14 days showed complete elimination of E. coli and S. typhimurium, which made the final effluent safe for open irrigation.

**Conclusion**

This chapter has shown that edible parts of tomatoes are safe from bacterial pathogens when wastewater or a solution containing pathogens is used as the nutrient medium. The effluent after passing through the hydroponics system was completely deprived of E. coli and Salmonella sp. between 7-14 days. The effluent after going through these systems (nutrient film technique and water culture) can be used safely for further irrigation. However, it has to be noted that the safety depends on the system hygiene.
References


