Particle size distribution as a useful tool for microbial detection

A. Chavez, B. Jimenez and C. Maya
Engineering Institute. Group: Treatment and Reuse, UNAM. Ciudad Universitaria, P.O.Box 70-472, Coyoacan 04510 Mexico City, D.F. (E-mail: AChavezM@iingen.unam.mx)

Abstract Worldwide, raw or treated wastewater is used for irrigation. However, this practice implies that the microbial content must be controlled. Unfortunately, detection techniques for microorganisms are costly, time consuming, and require highly trained personnel. For these reasons, this study used particle size distribution to measure the microbial quality of wastewater through correlations between the number or volume of particles and the concentration of fecal coliforms, Salmonella spp. and helminth ova. Such correlations were obtained for both raw and chemically treated wastewater. The best fit was the one for helminth ova, which applies for both the influent and effluent and also for all the coagulants involved. This technique allows the on-line quantification of helminth ova at a cost of US$3 and it takes only 5 minutes, instead of the US$70 and 5 days for the standard technique. With respect to the coagulants applied, their behavior is different only for particles smaller than 8 µm, and thus this value is considered as the critical size for this particular treatment. The best coagulant was the aluminium polychloride. In addition, this work establishes the distribution of COD, TSS, nitrogen, and phosphorous for particles smaller and larger than 20 µm.

Keywords Advanced primary treatment; agricultural irrigation; chemical enhanced primary treatment; helminth ova, particle size distribution

Introduction
Countries like Argentina, Brazil, Chile, India, Israel, Mexico, Saudi Arabia, and Tunisia use wastewater for agricultural irrigation, either with or without treatment (USEPA, 1992). However, there is a potential risk of transmitting gastrointestinal diseases, especially those caused by helminth ova (Cifuentes et al., 1993). For this reason, the World Health Organization (WHO, 1989) considers essential their detection and removal in irrigation water. The problem of gastrointestinal diseases is more severe in developing countries since the pathogenic levels in wastewater are very high as a result of public health problems. As an example, Jimenez et al. (2001) reported concentrations in wastewater of fecal coliforms, Salmonella spp. and helminth ova of 10^7–10^9 MPN/100 mL, 10^6–10^9 MPN/100 mL, and 6–93 ova/L, respectively. In contrast, values reported for the United States are 10^3 to 10^5 MPN/100 mL for fecal coliforms, 10^2 to 10^4 MPN/100 mL for Salmonella, and 1 to 8 ova/L for helminth ova (USEPA, 1992). In Mexico, it is estimated that at least 108 m^3/s of wastewater are used in the irrigation of 254,000 ha. For that reason, there is a standard that limits the concentration of fecal coliforms to less than 1,000 MPN/100 mL in all the cases and, additionally, helminth ova must be less than 1 ova/L for crops eaten uncooked, and less than 5 ova/L for the rest of the crops. Nonetheless, to meet the standard it is required to have the analytical techniques for those two parameters. Commonly, these techniques are expensive, complex and time consuming, making harder to measure these parameters and, moreover, the control and optimization of the treatment processes becomes a difficult task.

When measuring microorganisms, it has been forgotten that they are particles with different sizes. Fecal coliforms, traditional pollution indicators, have a size between 0.7 and 1.5 µm (Jawetz et al., 1995) while helminth ova range in size from 20 to 80 µm (Ayres,
1989). Considering their size and characteristics, it is possible to assume that the microbial content (free or associated to suspended solids) may be evaluated through the determination of the particle size distribution (PSD), as long as an adequate technique is available.

To determine the amount and size of the particles in wastewater, Allen (1997) and UBC (1999) propose different techniques. The choice of any of them depends on the physicochemical characteristics of the water, the particle size to be determined, the time required to get the results, and the equipment cost. One of the most recent and promising techniques, based on its versatility to provide information on the number and volume of the particles, is the one based on the Coulter principle (Allen, 1997). It measures particles ranging in size from 0.06 to 200 µm in about 5 minutes and at a cost of US$3 per sample. In addition, it provides information regarding the projected area of the particles, which is important for disinfection purposes.

As background for this study, Neis and Tiehm (1997) developed a correlation for raw wastewater and a secondary effluent between the fecal coliforms concentration and the number of particles, given by the equation 0.166 x number of particles (mL/L) + 4225. The authors indicate the usefulness of such equation without describing its validity for other cases. On the other hand, in drinking water, Larigies et al. (2001); and Elvers (1999) and Hall and Croll (1997) in Broadwell (2001), correlated the concentration of Giardia murris (3–9 µm) and Cryptosporidium parvum (2–5 µm) with the number of particles. The resulting equation is used, according to the authors, to design filters. Considering the importance of reusing wastewater for agricultural irrigation in Mexico, and to favor the compliance with the National Standard (1996), this study focus on measuring the microbial quality of wastewater through correlations between the number or volume of particles and the concentration of fecal coliforms (0.7–1.5 µm), Salmonella spp. (1.5–4 µm) and helminth ova (20–80 µm) and to determine the distribution of diverse parameters of water quality (COD, TSS, nitrogen, phosphorous and microorganism) and the particles smaller and larger than 20 µm.

Methodology

Tests were performed with raw wastewater generated in Mexico City during the months of May and August, 2001. The water was taken from a channel that transports from 30 to 40 m³/s of wastewater. On each sampling, 50 L were taken. The samples were analyzed before and after coagulation–floculation in a jar test apparatus. Coagulants applied were aluminium sulphate, and three aluminium polychlorides with different basicities (low, medium-high, and high) with the purpose of defining the effect of each of them on the resulting correlations. The treatment applied simulated an advanced primary treatment (APT) or Chemical Enhanced Primary Treatment (CEPT) which according to Harleman and Muczott (1999), and Jimenez and Chavez (1997) is applicable to treat wastewater destined to agricultural irrigation. Mixing conditions used were: 463 s⁻¹ during 15 s for coagulation and 58 s⁻¹ during 5 min for flocculation (Chávez and Jiménez, 2000). Each coagulant was used together with an anionic polymer of high molecular weight as flocculant. Samples were taken 5 min after sedimentation. Parameters analyzed in raw and treated wastewater were total suspended solids, turbidity, N-NH₃, P-PO₄, fecal coliforms, Salmonella spp., and helminth ova according to the Standard Methods (APHA, AWWA, WEF, 1995) and USBPA (1992). Particle size distribution was performed with a Coulter Counter–Multisizer™ II with 30, 100 and 200 µm (Ø) tubes. The equipment was programmed to obtain the number and volume of particles in four size ranges: (a) 0.75–1.5 µm; (b) 1.5–8 µm; (c) 8–20 µm; and (d) 20–80 µm. Ranges a, b and d correspond to the size of fecal coliforms, Salmonella spp. and helminth ova, respectively. To analyze the samples, they were diluted to 10% in 100 mL of an electrolytic solution. Each sample was analyzed by triplicate.
Results and discussion

Influent characteristics

Wastewater from Mexico City showed an average particle content of $2.3 \times 10^{13}$/m$^3$, with sizes ranging from 0.06 to 80 µm (Figure 1). From them, 84% were larger than 1.5 µm, 12% were between 1.5 and 8 µm, and only 4% were larger than 8 µm. Based on these characteristics and according to Levine (1991), the particles are mostly colloidal and supracolloidal and they require their agglomeration to be sedimented. With respect to the volume of particles, the average value was 1,436 mL/m$^3$. In most of the cases, 50% of such volume corresponded to particles smaller than 8 µm while the rest of them were larger (Figure 1).

Effect of the type of coagulant on particle removal

First, it was found that coagulation–flocculation removes up to 1.8 logs of particles (from $2.3 \times 10^{13}$ to $4 \times 10^{11}$ particles/m$^3$). This is equivalent to a volume reduction of 91% (from 1,436 to 124 mL/m$^3$). The only difference among coagulants is seen for particles smaller than 8 µm (Figure 2). Thus, coagulants behave on a similar way for particles larger than 8 µm since they represent only a small fraction of the total (4% in number). On this regard, the coagulant with a medium-high basicity was the most efficient as it separated more of the smaller particles (0.6 logs of particles smaller than 8 µm) and, consequently, it exhibited higher removals for fecal coliforms (1.38 logs) and Salmonella spp. (2.4 logs). The less efficient coagulant was aluminium sulphate because it only removed 0.76 and 1.2 logs of fecal coliforms and Salmonella spp., respectively. In addition, it only removed 0.3 logs of particles smaller than 8 µm. Based on the small differences in the behavior of the coagulants for small particles, it is considered that 8 µm is the critical removal size for this type of water and it also might be for the coagulation–flocculation process in general.
Figure 2 Percentage of particles in the effluent of the different treatments, based on their size

Particle content and correlation with microorganisms

_Helminth ova_. As observed in Figure 3, the helminth ova content is related to the volume of particles with sizes between 20 and 80 μm. That is not the case with the number of particles because of the magnitude of the values found. The correlation for this parameter may be expressed by the following equation:

\[
\text{Helminth ova (ova/L)} = 0.15 \text{ (volume of particles 20–80 μm, mL/m}^3\text{)}; R^2 = 0.98
\]  

(1)

This equation is valid for both the influent and the effluent and for the different coagulants tested, as the equations for each of them are similar. However, it is expected that the equation is valid only for the wastewater studied and it must be determined in other cases. From Figure 3, it may also be concluded that to achieve a helminth ova concentration on the effluent below 1 ova/L, there must be a maximum volume of particles with sizes between 20 and 80 μm of 8 mL/m³.

_Fecal coliforms_ and _Salmonella spp_. In this case, due to the high concentrations found (10⁵ to 10⁶) the equations that best fitted the results correlated the number of particles and not the volume for all the coagulants tested and raw and treated samples. The resulting equations were (Figure 4):

\[
\text{Fecal coliforms in Log(MPN/100 mL)} = 1.4277N - 5.1586, R^2 = 0.71
\]  

(2)

\[
\text{Salmonella spp. in Log(MPN/100 mL)} = 1.1159N - 3.3751, R^2 = 0.82
\]  

(3)

where \(N\) is the number of particles in log/100 mL.

Since the effluent has a great number of particles smaller than 8 μm (around 94%), most of the bacteria remain in the system, even after the treatment. In fact, coagulation–floculation only removes 0.76–3.8 logs of fecal coliforms, and 1.2–2.3 logs of _Salmonella spp_. These low efficiencies and the high microbial concentration in raw wastewater limit the applicability of the equations in the practice, as it only applies to this type of water with 10⁵ to 10⁶ bacteria. This means that the correlation must be updated when water quality changes.
Based on Eqs (1) to (3), it is possible to estimate to a certain degree the concentration of fecal coliforms and *Salmonella*, both in the influent and effluent of the analyzed water. The main advantage is that this technique needs only close to 5 minutes to get results, instead of the 24 hours needed to determine bacteria or 5 days for helminth ova. Besides, monitoring can be on-line, which will help in the control of the coagulation–flocculation process. On the other hand, the cost of particle analysis is 10 times lower than that for analyzing fecal coliforms and *Salmonella* spp. (30 USD each), and 23 times cheaper than helminth ova (70 USD). Another advantage is the easiness to operate a particle counting equipment, compared to the tedious microbial analyses. However, this technique has the disadvantage of needing to determine the correlations for different effluents and for other processes.

**Correlation between the suspended solids and particle size**

When determining the correlation between the particles and suspended solids, the equation that best fitted the results was the one that involves the volume of particles:

\[
\text{TSS in mg/L} = 0.22 \times \text{(volume of particles, mL/m}^3\text{)} + 8, R^2 = 0.93
\]  

(4)

It should be mentioned that Eqs (1) and (4) are not related since they were obtained using...
different particle size intervals. In the case of Eq. (4), the correlation was obtained for particles between 0.06 and 80 μm, while Eq. (1) used only particles from 20 to 80 μm as they correspond to the size of helminth ova. Nonetheless, by using both equations it can be determined that an effluent concentration of 1 helminth ova/L requires a volume of particles (between 20 and 80 μm) of 8 mL/m³ which in turn corresponds to a TSS content of approximately 35 mg/L.

**Distribution of pollutants with particles smaller and larger than 20 μm**

Since the most important issue in agricultural reuse is the concentration of helminth ova in wastewater, one of the objectives of the treatment is their complete removal. However, by separating the 20–80 μm particles that include them, other compounds that are needed by the crops, such as nitrogen and phosphorus, could also be separated. For this reason, the distribution of other parameters with particles smaller and larger than 20 μm was analyzed. To perform such analyses, raw and treated wastewater was filtered through a 20 μm membrane. Then, the raw water and the filtrate were analyzed for the physicochemical and microbial parameters shown on Table 1.

**Microbial distribution.** According to Table 1, as was expected, particles smaller than 20 μm contain most of the bacteria, in both the influent and the effluent. In larger particles, a maximum of 12% of fecal coliforms and 15% of *Salmonella* were found. In contrast, all the helminth ova found were within particles larger than 20 μm.

**Distribution of COD, nitrogen and phosphorus.** In raw wastewater, particles smaller than 20 μm contain 40% of the nitrogen and 95% of the phosphorus, which means that by removing these particles, most of the nitrogen and only a fraction of the phosphorus will be removed. In this case, phosphorus is present in soluble form or linked to small particles. These results are similar to those reported by Marquet (1999), Odegaard (1992), and Thiem et al. (1999). In contrast, particles smaller than 20 μm contribute to COD with 50% in the influent and 70% in the effluent, therefore about 50% of the oxygen demand is removed by the coagulation–flocculation process.

**Distribution of TSS and turbidity.** Between 71 and 90% of the suspended solids, and 38 to 70% of the turbidity are caused by particles larger than 20 μm. This means that not all the turbidity is produced by the suspended solids contained in those particles.

**Table 1** Physicochemical and microbial quality of raw and treated wastewater and their association to particles smaller and larger than 20 μm.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Raw wastewater</th>
<th>Treated wastewater</th>
<th>Removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean concentration</td>
<td>% of concentration</td>
<td>Mean concentration</td>
</tr>
<tr>
<td></td>
<td>&lt; 20 μm</td>
<td>&gt; 20 μm</td>
<td>&lt; 20 μm</td>
</tr>
<tr>
<td>Fecal coliforms, MPN/100 mL</td>
<td>2.4 x 10⁹</td>
<td>88–99</td>
<td>1–12</td>
</tr>
<tr>
<td><em>Salmonella</em> spp., MPN/100 mL</td>
<td>3.1 x 10⁹</td>
<td>85–98</td>
<td>2–15</td>
</tr>
<tr>
<td>Helminth ova, ova/L</td>
<td>7.36</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>P-PO₄, mg/L</td>
<td>5.2</td>
<td>40–95</td>
<td>5–10</td>
</tr>
<tr>
<td>N-NH₃, mg/L</td>
<td>16.5</td>
<td>40–95</td>
<td>5–60</td>
</tr>
<tr>
<td>COD, mg/L</td>
<td>350</td>
<td>57–77</td>
<td>23–43</td>
</tr>
<tr>
<td>TSS, mg/L</td>
<td>360</td>
<td>10–29</td>
<td>71–90</td>
</tr>
<tr>
<td>Turbidity, NTU</td>
<td>233</td>
<td>33–62</td>
<td>98–70</td>
</tr>
</tbody>
</table>

* Removal in logs
Based on these results, by separating all the particles larger than 20 µm, all the helminth ova, and up to 43% of COD, 60% of ammonia nitrogen, and 10% of the phosphorus will also be removed. To achieve this, more than 71% of the suspended solids must be eliminated. This supports the importance of the coagulation–flocculation in the treatment of wastewater used for agricultural irrigation, since, by separating mostly solids and not the soluble fraction or very small particles, the concentration of pathogens is reduced and organic matter and nutrients are kept in the water.

Conclusions
It is possible to correlate the particle size distribution with the microbial quality of the wastewater. Nonetheless, the obtained equations may be valid only for the waste water and effluent used (in this case, high content of helminth ova and fecal coliforms).

Particle size distribution can be used to monitor helminth ova concentration at the influent and effluent on-line, in 5 minutes and at a cost of US$3 instead of using grab samples, 5 days and 70 USD.

Fecal coliforms and Salmonella spp. can also be monitored using particle size distribution, but the equations presented only applied to certain concentrations (10^4 to 10^8).

Last, it was found that the wastewater analyzed contained 10^{13} particles/m^3 in a volume of 1,436 mL/m^3. In number, 96% of the particles are smaller than 8 µm and basically include all the fecal coliforms and Salmonella spp. In fact, this may be considered as the critical value for all the coagulants since the difference in their performance is only appreciated for particles smaller than that value.

References


