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COMMENT

Comments on: "Virus adsorption in a complex system: an experimentally designed study" by F. Quignon, F. Thomas, C. Gantzer, A. Huyard and L. Schwartzbrod *Wat Res* **32**(4), 1222–1230 (1998).

This is an unusually ambitious paper. As indicated by its title, it has *two* major purposes, of approximately equal importance. One is to determine, using polioviruses in laboratory aqueous environments similar to those found naturally, how virus adsorption onto clay particles depends on the chemical conditions, which is information with numerous potential applications to water and wastewater engineering. The other is to convince water and wastewater engineering researchers that experiments designed according to principles derived from combinatorial mathematics can determine interaction effects in multiparameter systems that would be overlooked in less sophisticated experiments.

The authors have performed an excellent study and have presented a very clear description of the work. The following questions and comments are intended less as criticisms than as ways of clarifying our own understanding and perhaps that of other readers.

1. Were the Doehlert matrices chosen because they have some clear superiority over other types of combinatorial designs (e.g. other types found in the *CRC Handbook of Probability and Statistics*)? If so, what makes them superior?
2. Has mathematical research in the decades since Doehlert's papers in 1970 and 1972 turned up any newer experimental design principles that would be applicable to these types of experiment? Does the importance of the 'additional experiments' (the ones performed on combinations of parameters that were not part of the Doehlert matrix system) imply a need to find combinatorial designs that would be more comprehensive than the Doehlert matrices?
3. Has there ever been an effort (by virologists or other microbiologists, if not by water and wastewater engineering researchers) to observe viruses adsorbed on clay particles, using scanning electron microscopy or any other imaging technology? Would such imaging applied to particles formed under varying chemical conditions add insight to the kinds of observations recorded in this paper?
4. Do the results in this paper lead to any feasible recommendations for enhancing virus removal in tertiary treatment in wastewater treatment plants? As the authors probably know, microfiltration does an excellent job of removing cellular organisms, but its performance in removing viruses, which are far smaller than the pores of microfiltration media, is highly variable (Jacangelo *et al.*, 1995; Iranpour *et al.*, 1998a, b), and so it has been widely accepted that microfiltration must be followed by a final disinfection step, either chemical, using chlorine or ozone, or physical, using UV. These results raise the possibility of an alternative approach using virus removal by clay adsorption before microfiltration, with the need for final disinfection reduced or eliminated. Do the authors have any comment on this possibility?
5. Tables 3 and 4 show standard deviations of the results of the additional experiments, but there is no indication in Table 2 or Fig. 3 of the variability of the results from the experiments in the Doehlert matrix designs. Although these were all done only in duplicate, and therefore could not simply be fed into the conventional standard deviation formula, we note that each estimated virus concentration was obtained by a titration and counting method that allowed associating a confidence interval with it and that the confidence interval was reduced by a maximum likelihood test, all of this being computed by the methods of Maul (1991). Are we correct in concluding that it would have been possible to indicate a magnitude of uncertainty for these results?
6. Is it realistic to be concerned about the possibility of saturating the adsorption capability of clay samples after prolonged use for virus adsorption? Since the experimental methodology assumed that adsorption need not cause inactivation, and this was confirmed by the results, it seems possible that eventually the available adsorption sites on the clay particles would be filled, and additional viruses would pass through, or have an equivalent effect by displacing previously adsorbed viruses, in a manner somewhat analogous to the way that other filter media become saturated with removed material and must either be replaced or backwashed. As backwashing seems inapplicable in this context, a valu-

able use of the results of this paper might be to help understand the possibilities for manipulating the chemistry of an aqueous environment to promote inactivation of adsorbed viruses.

7. Along these same lines, we note that when a space of system responses to varying combinations of input parameters has been surveyed with sufficient thoroughness, the data may be used as inputs for a search for optimum combination, using dynamic programming. Is it realistic to try to use dynamic programming in this context? More specifically, if the Doehlert matrices are derived from the vertices of multidimensional cuboctahedrons, as indicated by the title of the Doehlert and Klee (1972) paper, do the authors know of any attempts to adapt the general strategy of dynamic programming to the particular geometry (edges, faces, etc.) of cubotahedra?
8. Is anything known about the mechanism of virus inactivation by tannic acid, such as by a 'tanning' effect on the coat proteins? Is the inactivating effect specific to tannic acid characteristic of a wider variety of organic materials found in aqueous environments? These questions are prompted by recalling that discussions of UV disinfection frequently mention that irradiation by light in the wavelength range 250–270 nm causes thymine dimerization in nucleic acids, and this is generally believed to be the most important mechanism of inactivation of microorganisms in these systems.
9. Does the type of virus make a difference in the results? For example, if similar experiments were done with coliphage viruses, is it likely that the results would be significantly different? Since water and wastewater engineering are not usually concerned with viruses that are not infectious to humans, repeating at least a few of these tests on coliphages would not directly add to predictions of the health and safety effects of various virus removal processes involving clay. On the other hand, since coliphages are now often used as substitutes for human viruses, because in certain respects their properties are similar, this test would serve as a test of this substitution, which would fit in with the authors' interest in influencing engineering research methodology.
10. How were the ranges of the parameters used in this study chosen? As the Doehlert matrices are independent of the specific conditions of any experimental study, it appears to us that the two-decade ranges of virus concentration, ionic strength, organic material concentration and clay concentration in this paper were based primarily on past experience with natural aqueous environments. However, since we are interested in the possible applicability of these results to wastewater treatment, where values of some of these parameters may fall outside the ranges observed by the authors, we would like to know a little more about the reasons for the choices of ranges.

Let us close by repeating that this is an outstanding paper and that both the specific virus results and vocacy of combinatorial design methods for experiments on complex systems deserve careful attention from water and wastewater engineers.

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