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Detecting recalcitrant organic chemicals in water with microbial fuel cells and artificial neural networks

Scott T. King [a], Marc Sylvander [a], Mekhakhem Kheperu [b], LeeAnn Racz [a], Willie F. Harper Jr. [a],*

[a] Department of Systems Engineering and Management, Air Force Institute of Technology, 2950 Hobson Way, Wright Patterson Air Force Base, OH 45433, USA
[b] Spelman College, Department of Physics, 350 Spelman Lane, Atlanta, GA 30314, USA

HIGHLIGHTS

- Recalcitrant chemicals were detected with microbial fuel cells.
- We used three ways to parameterize the electrical signals.
- Test chemicals were associated with distinct current profiles.
- We accurately predicted chemical concentrations using ANNs.

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ABSTRACT

This study integrates artificial neural network (ANN) processing with microbial fuel cell (MFC)-based biosensing in the detection of three organic pollutants: aldicarb, dimethyl-methylphosphonate (DMMP), and bisphenol-A (BPA). Overall, the use of the ANN proved to be more reliable than direct correlations for the determination of both chemical concentration and type. The ANN output matched the appropriate chemical concentration and type for three different concentrations and throughout a wide range of stepwise tests. Additionally, chemicals dissolved in the acetate-based feed medium (FM) were accurately identified by the ANN even though the acetate masked the pollutants' effects on electrical current. The ANN also accurately revealed the identity of chemical mixtures. This study is the first to incorporate ANN modeling with MFC-based biosensing for the detection and quantification of organic pollutants that are not readily biodegradable. Furthermore, this work provides insight into the flexibility of MFC-based biosensing as it pertains to limits of detection and its applicability to scenarios where mixtures of pollutants and unique solvents are involved. This research effort is expected to serve as a guide for future MFC-based biosensing efforts.

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1. Introduction

Microbial fuel cells (MFCs) are bioelectrochemical devices that have been employed in water quality monitoring. MFCs consist of an anode and cathode; the former is typically immersed in water and exposed to an electron donor (e.g. an organic compound) while the latter is exposed to a terminal electron acceptor like oxygen (Logan, 2007). A permeable membrane separates the two chambers. Anode-respiring bacteria (ARB) grow on the anode, oxidizing chemical substrates and transferring electrons to the surface of the electrode. These electrons then flow through a wire across an external resistor and to the cathode where the terminal electron acceptor is reduced. This current can be measured and then correlated to water quality because ARBs are impacted by the constituents present in their growth environment. A number of previous studies have demonstrated that MFCs can be used to detect biodegradable chemicals dissolved in water (Kumlanghan et al., 2007; Feng et al., 2013a; Tront et al., 2008). Feng et al. (2013a) contributed to this literature by showing how MFCs can be used to distinguish and identify specific chemicals present in water samples. There is now a clear consensus supporting the idea that MFCs can detect biodegradable chemicals in water, and there is also a great wealth of information now available about the construction, start-up, and long-term operation of these devices (Logan, 2008). In principle, MFC-based monitoring of water is now possible at any scale.

Many of the chemicals that are of great concern in the water quality community are recalcitrant organics; chemicals that persist in the aquatic environment because they are not readily degradable (Ciputra et al., 2010; Maszenan et al., 2011). Notable examples include pesticides, solvents, plasticizers, gasoline additives, flame retardants, pharmaceuticals, and numerous other chemicals with properties (e.g. low

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* Corresponding author. Tel.: +1 937 255 3636x4528; fax: +1 937 656 4699.
E-mail address: willie.harper@afit.edu (W.F. Harper).

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solubility, high molecular masses) that make them resistant to degradation (Bhatt et al., 2007; Andreozzi et al., 2003; Focazio et al., 2008; Kit Chan et al., 2012). Water should be monitored for these chemicals in order to minimize or avoid exposing people to the associated toxicity. MFC-based water monitoring has never been accomplished for organic chemicals that are recalcitrant and this is especially challenging because MFCs work by utilizing bacteria that generate current. It is not clear that ARB can remain active while being exposed to chemicals that may cause inhibition. The effect of recalcitrant chemicals on MFC operations must be studied.

Correlating MFC outputs with the desired water quality parameter(s) can be complicated by a two primary factors. First, the output signals can vary due to changes in the composition of the water samples. For example, the pH or ionic strength can change or the target pollutant(s) may be mixed (and/or interact) with other compounds. Second, the MFC outputs may gradually change due to the evolution of the microbial ecology, which may or may not favor ARB (Lee et al., 2008; Lovley, 2008; Parameswaran et al., 2012; Rismani-Yazdi et al., 2013). Temporal output variations like this complicate the task of correlating MFC output with water quality. This is a key reason why artificial neural networks (ANNs) are employed in this research. An ANN is a network of computational models that resemble natural neurons. ANNs are inspired by human biological neural networks and are differentiated from other computer models for their ability to “learn” like the human brain. ANNs map the relationship between input and output data and can be adapted to changing patterns. These models correlate input and output data with a series of hidden layers, are trained with real data, and are then tested against a separate data subset. The highly flexible nature of ANNs allows the user to correlate several parameters simultaneously. Feng et al. (2013a, 2013b) successfully used MFC-generated electrical signals and ANN processing to both distinguish between four separate, readily biodegradable, simple organic substances and detect various chemical oxygen demand (COD) concentrations.

The objectives of this study are to

- correlate MFC data with the concentration and identity of three recalcitrant organic pollutants
- integrate ANNs into MFC-based biosensing for the identification and quantification of three recalcitrant organic pollutants
- determine the effect of the solvent on chemical identification via the ANN
- determine the effect of chemical mixtures on chemical identification via the ANN.

The three chemicals used in this work are aldicarb, dimethylmethylphosphonate (DMMP), and bisphenol-A (BPA). These chemicals are resistant to degradation and would be unlikely to serve as substrates for ARB (Khandaker and Young, 2000; Ike et al., 2000; McGrath et al., 1997) (see Appendix A). The hypothesis of this work is that ANN modeling will enhance MFC-based detection of these three compounds.

2. Materials and methods

2.1. Experimental overview

The overall strategy involved the injections of aqueous solutions of dissolved chemicals into the MFCs in order to generate a response in the form of electrical current. Each injection resulted in a single electric current profile (response peak) that was evaluated on the basis of six separate metrics. Those metrics were: peak height (PH, i.e. maximum charge), peak area (PA, i.e. area under the response charge), acceleration rate (AR, i.e. rate of increasing charge), subsidence rate (SR, i.e. rate of decreasing charge), 10-hour subsidence rate (10SR, i.e. rate of decreasing charge over a 10 hour period), and first moment (FrM, i.e. first moment about the y-axis). Supplemental information pertaining to metrics can be found in Appendix B. For each set of experiments, regressions were carried out. For example, each metric was plotted against chemical concentration to evaluate the correlations. Then, ANNs were trained and tested with the same data sets. Systematic testing was accomplished for the purpose of both quantifying the chemical concentration and identifying the chemical. Appendix C contains experimental results not shown in the main body of this paper.

2.2. Microbial fuel cell operation and configuration

Six single-chamber MFCs were operated as batch reactors in this study (Fig. 1). These devices were inoculated with activated sludge obtained from the Fairborn Water Reclamation Center in Fairborn, Ohio. A description of the materials used in the construction of these MFCs can be found in previous work (Feng et al., 2013a). All MFCs had an anode chamber volume of approximately 40 mL. Both 47 Ω (MFC #5, #6, #7 and #8) and 470 Ω (MFC #9 and #10) resistors were used. Electrodes were connected via a copper wire to a Keithley meter (Model 2750, Keithley Instruments) to monitor voltage outputs from the MFCs. Data collected by the Keithley meter was converted into tabular format via the ExceLINX program (Keithley Instruments) for analysis. The operating temperature was 21 °C. The MFCs were initially operated on acetate-based feed until stable current production was observed (see Appendix C, Fig. C7 for an example of a peak generated from acetate only).

2.3. Synthetic solutions

Four types of synthetic solutions were used for laboratory experiments. An acetate-based feed medium (FM) was composed of the following substrates: $C_2H_3NaO_2$, 430 ppm; NH_4Cl, 20 ppm; KH_2PO_4, 1360 ppm; K_2HPO_4, 200 ppm; $MgCl_2$, 250 ppm; $CoCl_2$, 20 ppm; $ZnCl_2$, 10 ppm; $CuCl_2$, 10 ppm; $CaCl_2$, 4 ppm; and $MnCl_2$, 10 ppm. Additionally, aqueous solutions for each of the three chemicals used in experiments (aldicarb, DMMP, and BPA) were created at different concentration levels. Aldicarb was purchased from Ultra Scientific in a solvent matrix form at a concentration of 100 μg/mL in acetonitrile. DMMP was purchased from Sigma Aldrich in a purum (≥97%) liquid form. BPA was purchased from Sigma Aldrich in a crystalline solid (97% purity) form. All water used in the composition of solutions was purified through reverse osmosis (RO).

2.4. Electrochemical testing

Systematic testing was accomplished with the chemicals over multiple ranges of influent concentrations via three experiment sets. The overall methodology involved the injection of the chemical solution for eight consecutive injections, an intermittent injection of standard FM, followed by eight consecutive injections of chemical solution for the next stage of testing. The intermittent injection of FM was introduced in order to supply the ARB with an adequate food source. This pattern proceeded until the experiment was complete. Voltage data was obtained from the Keithley Meter at two-minute intervals. Current was calculated using Ohm's Law. Intervals between injections were approximately 48 h. Two MFCs were used for each of the three experiment sets.

2.4.1. Quantification and identification testing

Both aldicarb and BPA were introduced at the concentrations of 800 ppb, 400 ppb, and 200 ppb. DMMP was injected at concentrations of 916 ppm, 458 ppm, and 229 ppm. Each chemical was tested at varying concentration levels so that: (1) charge outputs from a wider range of chemical concentrations could be observed; and (2) the higher concentrations might better simulate a chemical spill event where MFC-based biosensing could be used as a decision support tool. During each experiment, the peak metrics were recorded for

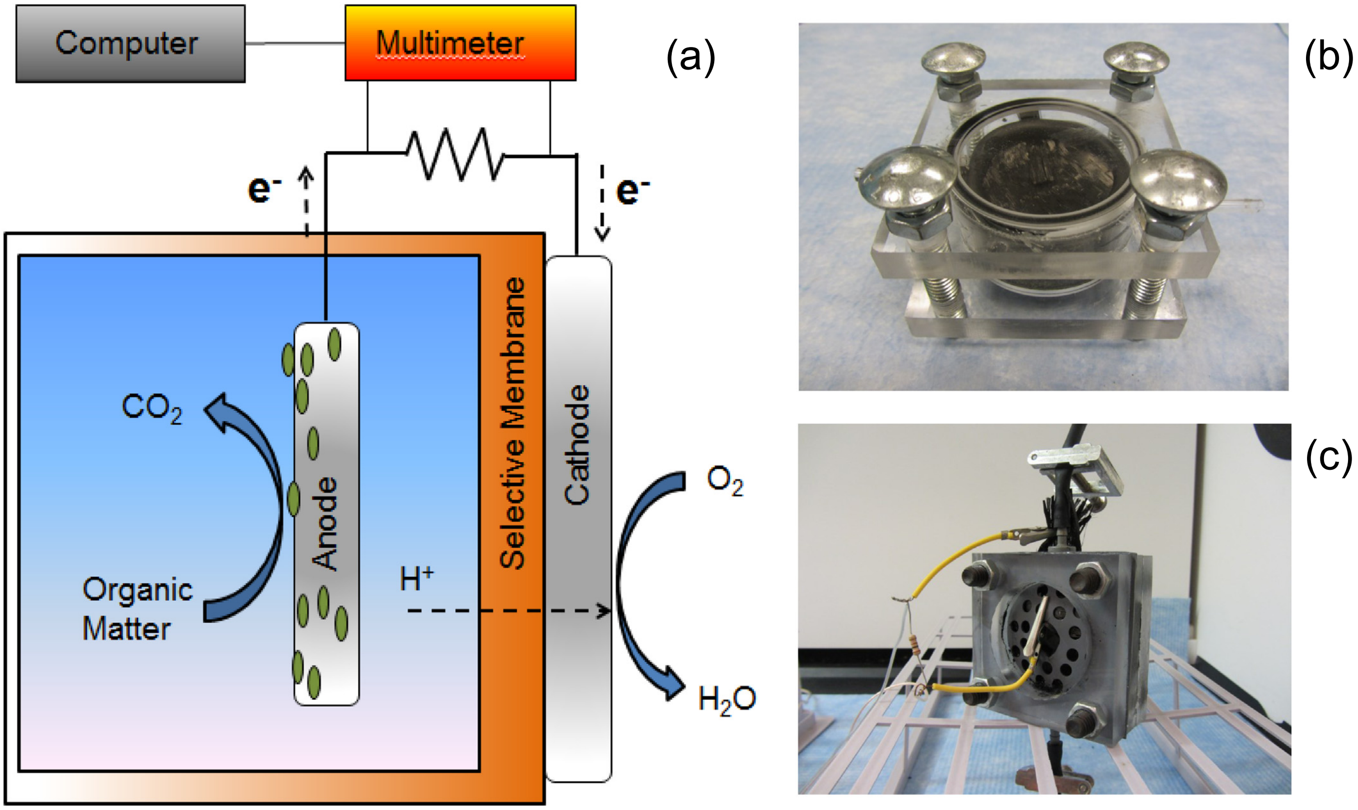

Fig. 1. (a) basic components of a single-chamber MFC; (b) view of anode chamber and carbon fiber anode; (c) configuration of electrode connections and external resistor.

subsequent analysis and ANN development (see Appendix C, Table C1 for examples of metric values retrieved for each chemical).

2.4.2. Solvent effect testing

Solvent effect experiments were conducted to determine if the presence of the FM as a solvent, as opposed to RO water as a solvent, would affect how well chemicals could be identified. Since the FM promotes microbial activity and the exogenous transfer of electrons, increased microbial activity could either enhance the responses making them more easily discernible or serve to mask any effect that the chemical had on the ARB. During these tests only one concentration per chemical was used in both its aqueous and FM form. For consistency, the maximum concentrations used in the quantification and identification set was selected for use.

2.4.3. Mixture testing

These tests were carried out to determine how well chemical mixtures could be identified with MFCs and ANNs. For aldicarb and BPA an aqueous concentration of 800 ppb was used and for DMMP an aqueous concentration of 916 ppm was used. These concentrations were kept constant during the tests in order to simplify the scope of the experiment. Mixtures used the same concentrations for each respective chemical. The testing procedure involved eight consecutive injections of each mixture (aldicarb/BPA, aldicarb/DMMP, and DMMP/BPA) with an intermittent injection of the FM. Then, each chemical was introduced individually for eight consecutive injections with an intermittent injection of the FM.

2.5. Artificial neural network development

The ANN was developed to interpret the electrical signals generated by the MFCs. In this study a customized, feed-forward network with one-way connections between the input layer, hidden layer(s), and output layer was developed and tested. The ANN functioned through a three-step process of training, validation, and testing. First, the ANN was trained with 80% (randomly selected) of the data obtained during experiments described above. The Levenberg–Marquardt back-propagation algorithm was used during network training (Marquardt, 1963). During training, a system-generated output is compared to a desired output and the back-propagation algorithm corrects for errors in the system. Corrections update both weight and bias values. Training continues until the error is minimized. Second, the ANN used 10% of the data (also randomly selected) for validation. In this step, the performance of the network is estimated and stopping points for training are established. Third, the final segment (i.e. 10%) of the data set is used to test the ANN. During testing, the overall performance of the model is evaluated. Five tests were accomplished during every ANN run to evaluate the use of 1 to 5 hidden layers of artificial neurons. Input matrices for the ANN were arranged so that the utility of new metrics (10SR, FrM) and metrics used in previous research (PH, PA, AR, SR) (Feng et al., 2013a) could be evaluated. Supplemental information pertaining to the ANN can be found in Appendix B. MATLAB R2012b (MathWorks, Natick, MA) was the computational platform.

3. Results and discussion

3.1. Quantification and identification testing

3.1.1. Quantification

Fig. 2 displays the operating history for MFC #10, one of two MFCs used in this experiment set. The other biosensor, MFC #5 (see Appendix C, Fig. C1), produced similar results. Intermediate feedings with the standard FM are annotated in red. Intermediate use of the FM between the DMMP concentrations of 916 ppm and 458 ppm was not recorded

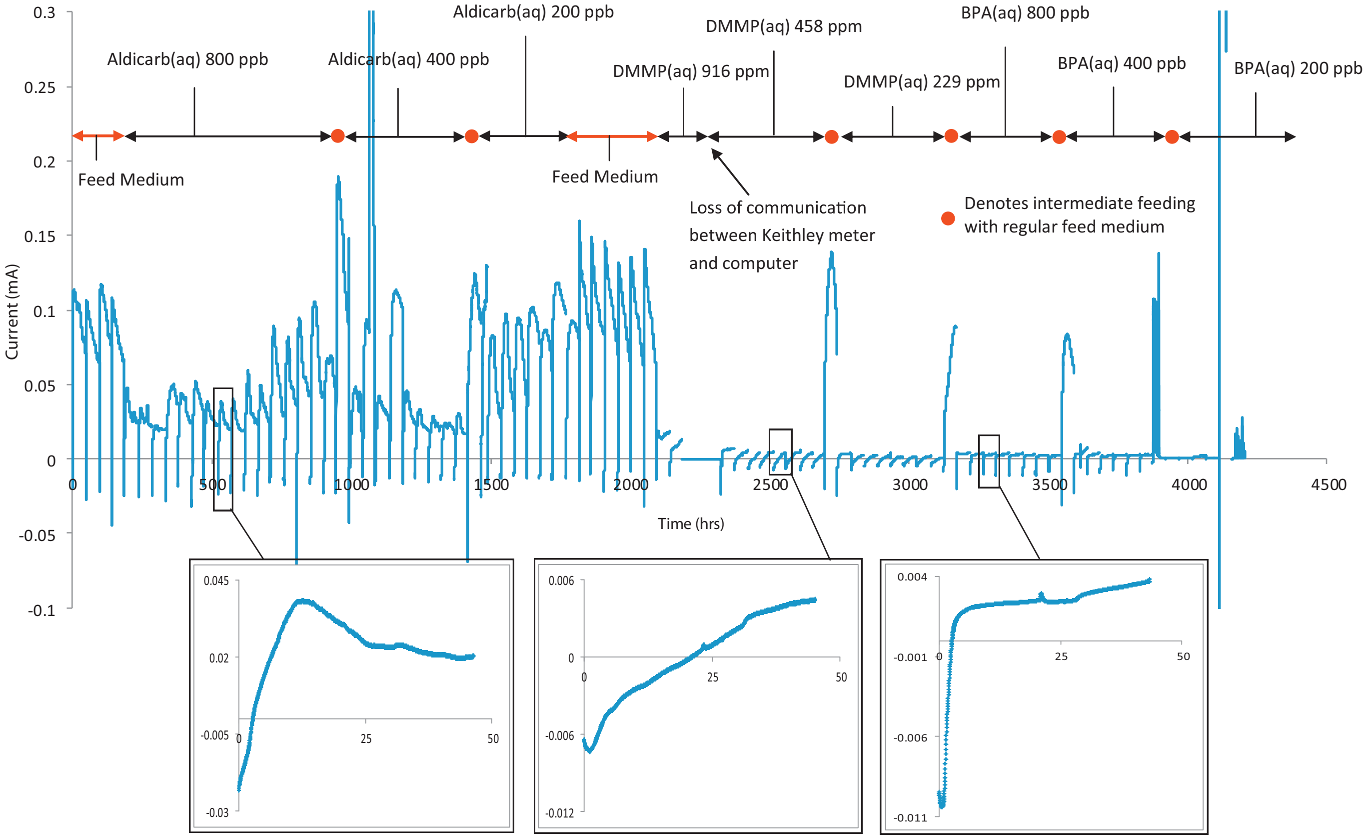

Fig. 2. MFC #10 operating history. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

due to a loss of communication between the Keithley Meter and the computer. Most of the electrical signals begin with an initial region showing negative current which may have been due to corrosion on the alligator clips. Qualitatively, all response peaks generally display an expected rise in current, but the peaks are highly dynamic, and the size and shape of the electrical signals are influenced by the chemical being added and its concentration. For example, when aldicarb was injected the current rose sharply followed by a slow, gradual subsidence rate. The aldicarb PH values were between 3.80E − 2 to 1.05E − 1 mA at 800 ppb, 4.30E − 2 to 1.13E − 1 mA at 400 ppb, and 8.30E − 2 to 1.18E − 1 mA at 200 ppb. The aldicarb signals were qualitatively different from DMMP signals, which had linear increases in current and were noticeably small (i.e. PH values were less than 1.90E − 2 mA). The BPA signals had a small increase in current followed by a longer horizontal plateau and the PH values were less than 4.00E − 3 mA. These results show that the three chemicals being studied had electrical signatures

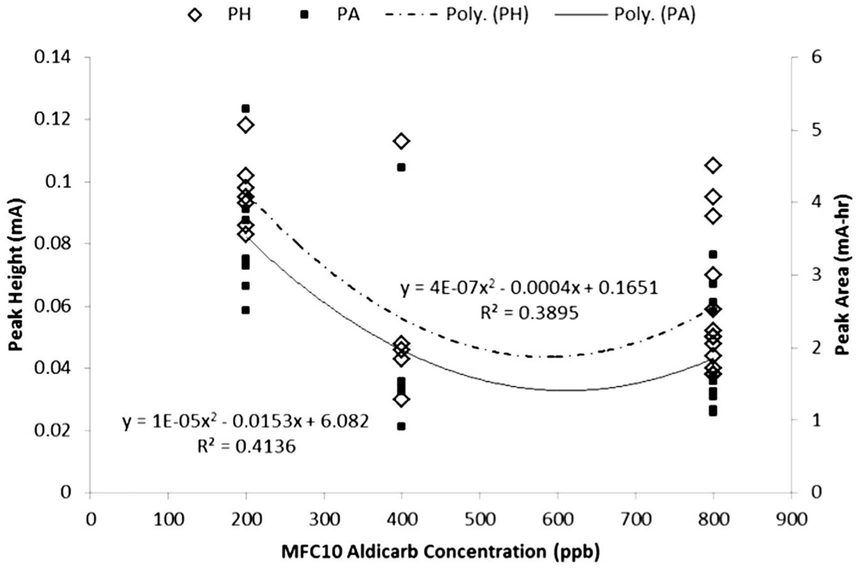

Fig. 3. PH and PA correlations with Aldicarb concentrations in MFC #10.

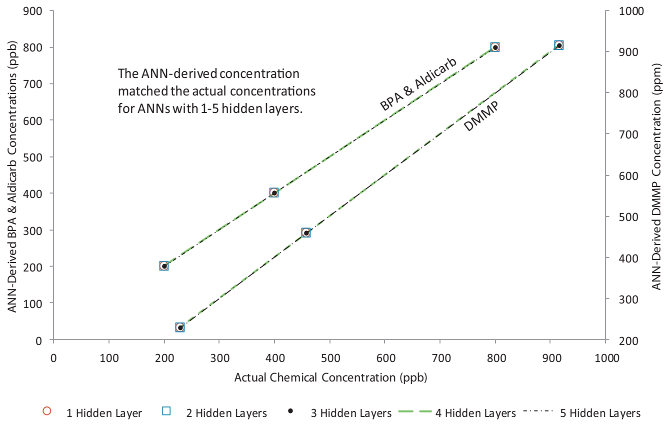

Fig. 4. ANN results for chemical quantification tests.

that were distinguishable. Fig. 2 also shows the effect of the intermediate acetate feedings. Acetate boosted the current PH to 8.30E − 2 mA or more, and because electrical current is caused by ARB, these data show that ARB colonies remained responsive throughout the experiment and in spite of being affected by organics that are not readily biodegradable. This result attests to the robustness of these ARB populations.

All recovered metrics in each category (PH, PA, SR, 10SR, AR, and FrM) were plotted against the measured concentrations of each respective chemical (e.g. Fig. 3). Overall, most regressions between metrics and concentrations resulted in non-linear correlations with low coefficients of determination (R^2) ranging from 0.02 to 0.90 (see Appendix C, Table C2). This shows that most of the metrics were not well-correlated to the chemical concentration present in the feed.

These data sets were used in the development of the ANN (see Appendix C, Tables C3–C4). The ANN-derived chemical concentrations were in agreement with the actual concentrations for each chemical tested (Fig. 4). The ANNs with up to five hidden layers generated model output that matched the actual chemical concentration. This result is a powerful illustration of the capabilities of ANNs. The trained model converted a group of metrics into meaningful water quality information; even though the electrical signals were temporally dynamic and each of the individual metrics did not directly correlate to chemical concentration. This result is a key reason that ANNs are useful for detecting recalcitrant chemicals. Furthermore, each of the input matrices ([PH, PA, AR, SR], [PH, PA, AR, 10SR], and [PH, PA, AR, FrM]) produced identical results, which shows the flexibility of ANNs, as well as the usefulness of the 10SR and FrM as quantitative metrics. Collectively, these results show that recalcitrant chemicals can be quantified using MFCs and ANNs.

3.1.2. Identification

The effect of the chemical types on the values of response metrics was evaluated to determine whether or not the raw data was suitable for the identification of chemicals. Fig. 5 presents an example for MFC #5 showing outlier box plots to help describe AR data ranges for all three chemicals that were tested. The box plot graphically presents the range of measured values. The central box line is the median value and the whisker lines extend up to 3 standard deviations away from the median. Outlaying data points lay outside of the range of the whisker lines. Fig. 5 shows that the range of AR values associated with aldicarb overlaps with the data ranges of DMMP and BPA. For example, an AR value of 0.007 mA/h is not unique to a particular chemical. The range of AR values for each of the three chemicals is not unique, which shows that AR values cannot be used to identify the chemical present in the MFC. We repeated this analysis for each metric for MFC #5 and MFC #10 and we found that no single metric took on unique values in the presence of the three chemicals (see Appendix C, Table C1 for the ranges of metric values retrieved for each chemical). No individual metric can be used to infer the identity of the chemical present in the MFC. However, a revised ANN was created where the specific chemical was the target of ANN prediction in the output layer instead of chemical concentration. The ANN-derived chemical identities matched the actual identities for 1–5 hidden layers and for every combination of metric input tested for both MFC #5 and MFC #10 (see Appendix C, Fig. C6). This time the data set encompassed all metrics for the three chemicals and over multiple concentration ranges. The fact that the ANN was able to help identify chemicals across the entire data set is particularly noteworthy in light of the diverse and overlapping ranges of metric values. This provides evidence that multi-parameter ANN modeling can differentiate between chemical types. Furthermore, since the ANN was able to accurately sort out the chemical type amongst the broad range of data inputs, it is conceivable that ANN modeling can first be done to determine the chemical type and then determine the concentration of the chemical within that type.

3.2. Solvent effect testing

It is important to try to understand how difficult it may be to detect chemicals when they are in a more complex solvent. Therefore, we carried out experiments in which the chemicals were dissolved in our FM which included acetate, mineral salts, and trace metals, and we

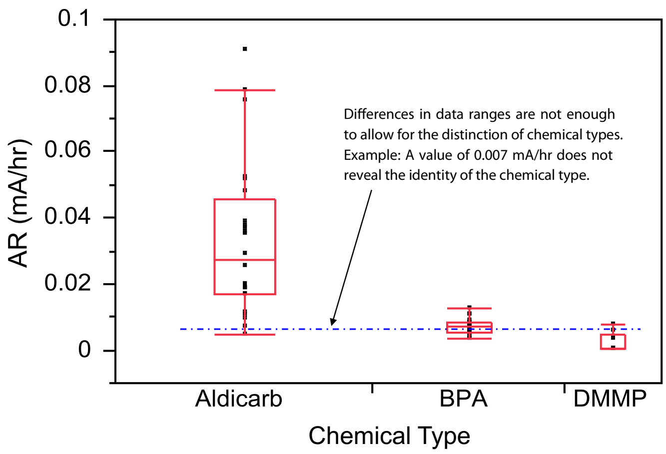

Fig. 5. The effect of chemical type on AR (mA/h) in MFC #5.

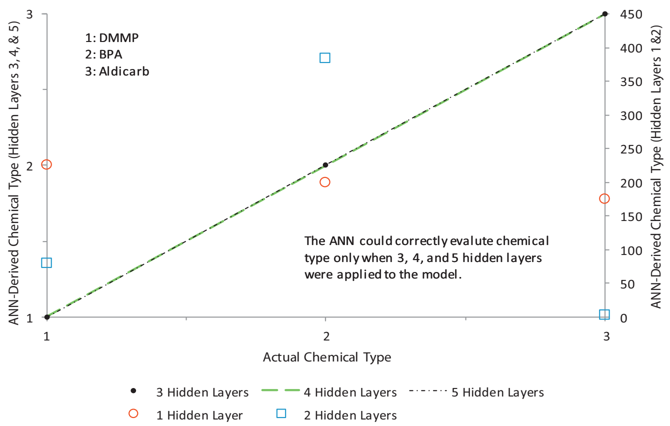

Fig. 6. ANN results for solvent effects testing using [PH, PA, AR, FrM] as the Input matrix for MFC #6.

compared the experimental results to others in which we dissolved the chemicals in water. The operating histories for the two MFCs (#6 and #7) used in this experiment set can be found at Appendix C (Figs. C2–C3). Qualitatively, the response peaks showed the sharp rise and gradual fall that were characteristic of the other experiments. We found that the electrical signals generated in the presence of FM were generally greater than those that were generated in the absence of FM. For example, in MFC #7 the DMMP(aq) peaks had PH values <3.30E−2 mA while DMMP(FM) peaks had PH values of up to 1.45E−1 mA. BPA(aq) peaks had PH values of <1.90E−2 while BPA(FM) had PH values up to 1.63E−1 mA. Aldicarb(aq) had PH values of up to 1.43E−1 mA while aldicarb(FM) had PH values of up to 2.59E−1 mA. Similar results were observed for MFC #6. The charge profiles with FM as the solvent show similarities in both size and shape (see Appendix C, Fig. C7). The similarities between the electrical signals suggest that current production is driven by the presence of the acetate-based FM and not the presence of the three target chemicals. These results also illustrate the potential difficulty of detecting industrial chemicals when they are in the presence of a readily degradable substrate such as acetate.

The ANN was used to attempt to distinguish the three target chemicals when they were introduced as a solute in the FM. All metrics (PH, PA, AR, 10SR, SR, and FrM) were used in the development of the ANN. The data sets used for ANN development can be found at Appendix C (Tables C5–C6) and it is important to note that the only data used to develop this ANN was retrieved from the MFCs used for solvent effects testing (i.e. MFC#6 and MFC#7). Fig. 6 shows the results from MFC #6 when the matrix [PH, PA, AR, FrM] was used as the input. As can be seen in the figure, the ANN-derived chemical identity matched the actual chemical identity for all three chemicals when three, four, or five hidden layers were used in the ANN. The same result was obtained for MFC #7 (data not shown). At least three hidden layers were needed to create the correlations required to distinguish the response peaks in this experiment. These results show that the ANN could be used to identify chemicals that are present in a more complex water matrix.

3.3. Mixture testing

Experiments were carried out to determine whether mixtures of the three chemicals could be distinguished from individual chemicals. The operating histories for the two MFCs (#8 and #9) used in this experiment set can be found at Appendix C (Figs. C4–C5). Many of the measured signals were highly dynamic and a several peaks lacked the well-organized structure that is needed to facilitate the retrieval of response metrics. In MFC #8 the aldicarb/BPA signals had PH values that were between 2.60E−2 and 6.30E−2 mA. Only one aldicarb/DMMP peak with a PH of 5.20E−2 mA was recovered due to a loss of communication with the computer. The DMMP/BPA signals were relatively stable in size and shape and had PH values that fluctuated around 2.00E−2 mA. Three of the DMMP (only) peaks resembled the DMMP/BPA peaks but not the aldicarb/DMMP peak. The aldicarb (only) peaks were highly variable and had larger PH and PA values than the aldicarb/BPA or aldicarb/DMMP peaks. The BPA (only) peaks had relatively consistent size and shape while being qualitatively different from aldicarb/BPA or DMMP/BPA peaks. An ANN was developed using only the data collected with chemical mixtures, but since not all peaks were useful for generating metric information, only 51% of the response peaks for MFC #8 and 47% of the peaks from MFC #9 were used in the ANN. A total of 25 peaks were evaluated in the data set for MFC #8 and 23 were in the data set for MFC #9. These data sets can be found at Appendix C (Tables C7–C8). The ANN correctly matched all mixtures and individual chemicals using all five hidden layers and for each set of input matrices tested (Appendix C, Fig. C8). Chemicals can be identified with an ANN even when they are present in a mixture and when the electrical signals are dynamic in nature. Future works should seek to collect enough data with chemical mixtures to better understand the effect of chemical mixtures on ARBs.

4. Conclusions

This study has provided an evaluation of the use of MFC-based biosensing for the detection of three recalcitrant organic pollutants. The MFCs produced electrical signals that were parameterized with several quantitative metrics, and these metrics could not be directly correlated to the identity or concentration of the three target chemicals. ANN processing of the signal metrics permitted accurate determination of both the concentration and identity of the industrial chemicals. ANNs were also useful in identifying chemicals that were dissolved in an acetate-based FM and for samples in which the target chemicals were mixed together. The ANN modeling approach was successful with well established signal metrics (e.g. PH and PA), and it also worked when trained with new signal metrics (i.e. 10SR and FrM) that had not been used previously. This study proves that MFC-based biosensing, when used in conjunction with ANNs, can successfully be applied to the detection and quantification of organic pollutants that are not readily degradable in MFCs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2014.07.108.

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