**MS4. Establishing a protocol for the experimental evaluation of the NUTRISENSE FH with the ISE operating online**

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The aim of this protocol is to experimentally test and validate the ability of NUTRISENSE, as controlling software of a fertigation system connected on-line with ion selective electrodes (ISEs), to optimize nutrient supply in closed hydroponic systems. This will be attained by automatically readjusting the composition of the replenishment nutrient solution whenever fresh nutrient solution is prepared by the fertigation head and sent to the crop through a drip irrigation system. More specifically, the following procedure is applied.

The on-line version of NUTRISENSE uses a series of algorithms based on mass balance models to calculate and control in real time the injection rates of liquid stock solutions to the mix of fertigation effluents (drainage solution) and water. For each nutrient, the variable quantity is its concentration in the drainage solution to be recycled, which is measured in real time by the ISE. In the NUTRISENSE project, a kit of eight electrodes purchased from CleanGrow, UK is used to measure in real time the concentrations of eight macroions in the drainage solution to be recycled. These macroions are $K^+$, $Ca^{2+}$, $Mg^{2+}$, $NH_4^+$, $Na^+$, $NO_3^-$, $H_2PO_4^-$ and $Cl^-$. Using data from a huge database (MS8), input data by the user, and the measurements of the ISE, NUTRISENSE automatically calculates in real time the injection rates of all fertilizers needed to prepare a NS with optimal composition. The efficiency of the online version of NUTRISENSE to improve the nutrient supply compared to the state of the art is depending on the fidelity of the ISEs.

To experimentally validate the efficiency of NUTRISENSE when connected on-line with ISEs through a fertigation system, the following steps will be followed.

1. A greenhouse plant species is cultivated in eight independent closed soilless cultivation units allocated into two groups with four units per group (2×4). Each group of four hydroponic units is treated differently concerning the supply of nutrient solution (NS). Thus, two different treatments concerning the management of nutrient supply are applied, with four replications per treatment.
2. In the first group of hydroponic units (1st treatment), a standard NS for the particular crop species is supplied, and its composition is readjusted every month through NUTRISENSE, based on a chemical analysis of the drainage solution.
3. In the second group of hydroponic units ($2^{nd}$ treatment), the composition of the supplied NS is automatically readjusted whenever a new nutrient solution is prepared and supplied to the crop using a version of NUTRISENSE that controls the operation of a fertigation system and is on-line connected with ISEs. Thus, the macroion concentrations measured in real time by the ISEs in the recycled drainage solution are transited in real time to NUTRISENSE. Using these data, NUTRISENSE calculates in real time the relative injection rates of fertilizer stock solutions to the irrigation water to prepare a replenishment nutrient solution with optimal composition. As the process of nutrient solution preparation starts, the fertigation system injects stock solutions of individual fertilizers to the mix of water and drainage solution at the rates calculated by NUTRISENSE. The whole process is fully automated.
4. All other information needed for the calculations of the relative injection rates (crop species, season of the year, mineral composition of the irrigation water used to prepare the NS, etc., are as in the

internet application of NUTRISENSE. The microion concentrations measured by the ISEs are $K^+$, $Ca^{2+}$, $Mg^{2+}$, $NH_4^+$, $NO_3^-$, $H_2PO_4^-$, $Cl^-$, and $Na^+$. Based on these data, the concentration of $SO_4^{2-}$ is estimated based on the electrochemical balance between anions and cations.

5. The version of NUTRISENSE connected on-line with ISEs calculates injection rates of individual fertilizers (e.g., KNO3, NH4NO3, KH2PO4, MgSO4, etc.). Therefore, to automatically readjust the composition of the replenishment nutrient solution in a closed hydroponic system using NUTRISENSE on-line with ISEs, eight stock solutions (SSs) are available and injected to the blend of irrigation water and drainage solution, as follows:

5.1. Calcium nitrate [$Ca(NO_3)_2$]
5.2. Potassium nitrate [$KNO_3$]
5.3. Ammonium nitrate [$NH_4NO_3$]
5.4. Magnesium sulfate [MgSO4]
5.5. Monopotassium phosphate [$KH_2PO_4$]
5.6. Potassium sulfate [$K_2SO_4$]
5.7. Magnesium nitrate [$Mg(NO_3)_2$]
5.8. Iron Chelate [Fe-EDDHA or Fe-DTPA]

In addition, there is one more tank containing nitric acid, which is injected at rates adjusted automatically by the fertigation system to achieve a target pH in the outgoing NS. The micronutrients are placed in the same tank with monopotassium phosphate.

6. At each irrigation event, NUTRISENSE records the concentrations of each particular nutrient: a) in the drainage solution, as measured by the ISEs; b) in the replenishment solution (net nutrient supply to the plant at each irrigation event) as calculated by NUTRISENSE; c) in the drip solution supplied to the plant (mix of drainage solution and replenishment NS). These data are automatically recorded at each irrigation event.
7. Every two weeks, samples of DS are collected and analyzed, and the obtained measurements are compared to those measured by the ISEs. Thus, the accuracy of the measurements conducted by the ISEs is checked.
8. The minimum duration of the experimental crops is eight weeks.
9. Samples of DS are collected also from the first treatment (standard NS) at each sampling date and used to determine the concentrations of the same nutrients and non-nutrient ions as in the second treatment (NUTRISENSE). However, in the standard NS treatment, these data are not used to modify the standard NS supply during the cropping period. They are used only to check which of the two treatments maintains the concentration of each macronutrient in the root environment closer to the target level for this particular macronutrient.
10. The evolution of the nutrient and non-nutrient ion concentrations over time in the DS, the replenishment NS, and the drip solution, are presented in figures for both treatments. In the same figures, the corresponding target concentrations of each particular nutrient, based on standard recommendations in the relevant literature, are also displayed, to compare the two strategies concerning their efficiency to optimize plant nutrition in a closed soilless culture system.