Effects of anaerobic root growth and nutrient limitation to the photosynthetic response and exudation of tomato and reed mannagrass plants.

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At the end of my practical period I would like to thank first of all my supervisor Jan Snel for his patience and good will.
I would like to thank all my colleagues for their support and help. I appreciate all that has been done for me.
Finally to all friends I am grateful forever.

Contents

Introduction

The natural production of carbohydrates from inorganic CO_2 with the contribution of chlorophyll and sunlight is called photosynthesis. It can be done by every living organism that contain chlorophyll and belong to the autotrophic organisms meaning those that produce their own food in contrast to the heterotrophic organisms that actually live on the carbohydrates produced by the autotrophic organisms.

Photosynthesis is the most primary way of absorbing of sunlight energy in the plants in the form of carbohydrates. These carbohydrates are being used by the plants to grow, to survive under stressful conditions, and generally for their daily energy needs until achieving the possibility to reproduce, which constitutes the core of their survival as a species.

The mechanism of photosynthesis

In the process of photosynthesis the energy is converted from sunlight energy into chemical energy that is used for the formation of carbohydrates, as known by the reaction:

$$\mathbf{6CO_2+6H_2O+light \rightarrow 6O_2 + C_6H_{12}O_6}$$

It is obvious that the synthesis of carbohydrates from CO_2 and H_2O is basically an redox reaction involving the reduction of CO_2. For this sequence energy and a provider of H^+ and electrons are needed. The energy comes from the sunlight. H^+ and electrons are derived from H_2O. Water in the presence of light and chlorophyll is been split into H^+ and OH^- in Photosystem II. O_2 and C that also are part of the synthesis come from CO_2. In the end the green pigment of chlorophyll is uniquely capable of converting the active energy of light into a latent form that can be stored (sugar) and used when needed. In Figure 1 we can see an example of the proccess step by step .

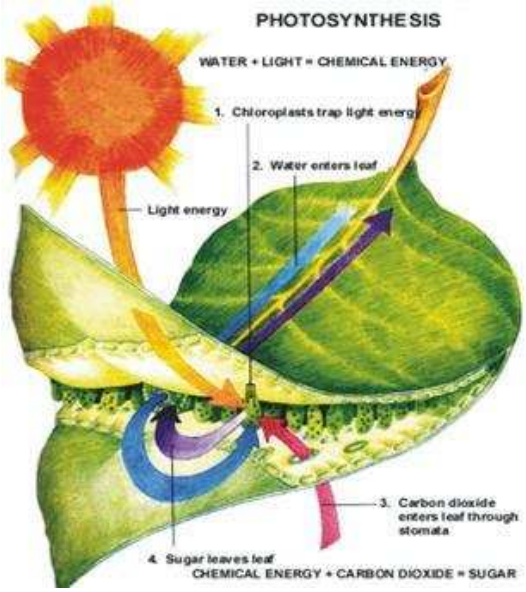

Figure 1. Photosynthesis is taken place on a leaf where the chloroplasts trap the light energy from the light while the absorbed water from the roots is carried to the leaves by the xylem. The same time carbon dioxide is obtained from air that enters the leaves through the stomata and diffuses to the cells containing chlorophyll. Finally CO_2 is been used for the creation of sugars

Photosynthesis as a sequence is done in the presence of light but it seems that there are other reactions also taking place that don't really need light. Those reactions, which we are going to talk about below, are called non-light reactions or "dark reactions" and they don't need light necessary to continue. Before we analyse the dark and light reactions lets first recall some short things about light.

The light

It is known that the visible light, the light that we see, is just a small part of the light that sun sends to earth. Light can be thought of in two different forms. The one is the form of discrete "packages" that are called photons or quanta. In the other form light is a wave with a given velocity and wavelength. Sunlight is mixture of (sun)rays with different wavelengths, which for the visible light, that matters in photosynthesis, is between 390 to 760 nm. In Figure 2 we can see the different wavelengths of light. When light comes in contact with an object a part of it is been absorbed and part is reflected. The wavelength of light that is reflected gives the impression of colour to that object. Therefore, as an example, things that reflect the green sunrays appear as green to us. If they absorb all sunrays, they appear black or if they reflect all light they appear as white and if they do not reflect or absorb then they appear as transparent (e.g. water).

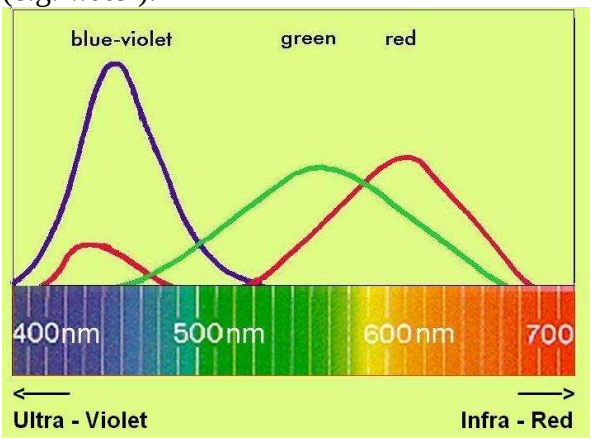

Figure 2. Wavelengths of light.

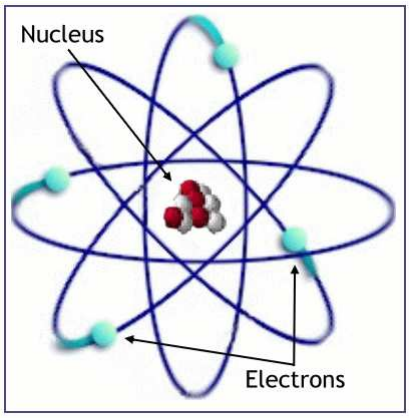

Figure 3. The Atom

The ability of an object to absorb light depends on the structure of its molecules and atoms. When a photon comes in contact with an atom of an object then an electron of that atom is been charged by the energy taken from the photon and reaches a higher energy stage. Afterwards, this electron returns to its normal stage. At that moment it releases the energy taken from the photon. This is either in the form of either i) heat or, ii) light of longer wavelength (fluorescence) or iii) a photochemical reaction. In the photochemical reaction of photosynthesis of plants the pigment chlorophyll is responsible for absorbtion of the light.

The photochemical reaction (light energy turns into chemical)

The light reactions occur in the thylakoid membrane of the chloroplasts. The light reactions take place in two clusters of pigment/protein complexes, known as photosystems I and II. Each photosystem possesses chlorophyll and several accessory pigments. These pigments help to make photosynthesis more efficient by absorbing different wavelengths of light.

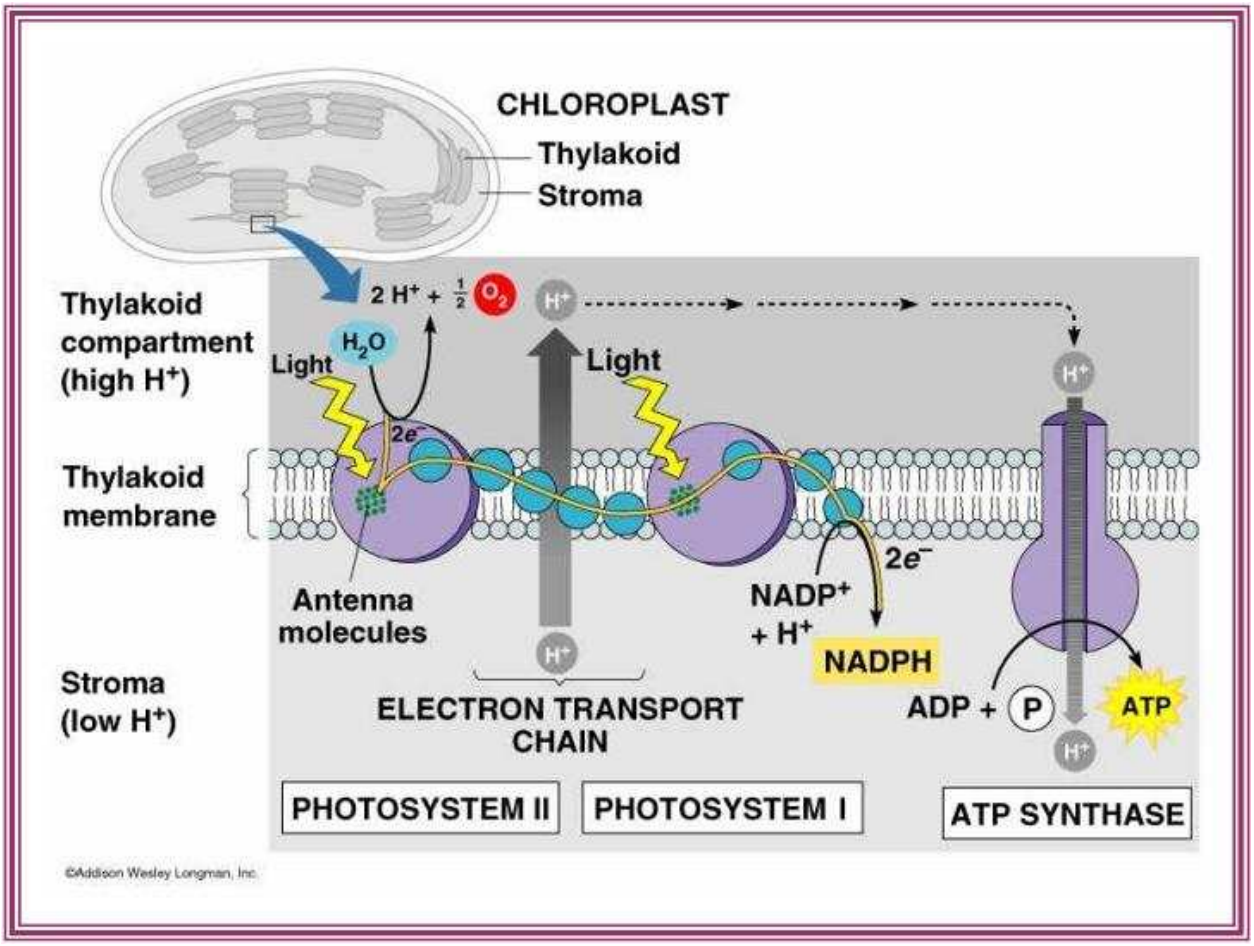

Figure 4. The light dependent reaction. The transportation of electrons where ATP and NADPH is been created.

When a photon gets in contact with a molecule of chlorophyll then an electron of chlorophyll is excited. The energy is transferred from the antenna molecules to special chlorophyll molecules where the photochemical reaction creates a chlorophyll cation and free electron. The electron transport chain makes it possible to use this electron for the reduction of NADP to NADPH with the help of H^+ taken from splitting of H_2O.

When light hits photosystem II, electrons gain more energy and are carried via a chain of electron-carrying proteins to photosystem I. When the light hits second photosystem, the electrons are moved again to a molecule of energy-rich NADP. The electrons needed to replace those removed from photosystem are provided by photosystem II. The H^+ produced by the splitting of water, supplemented with additional ions from the surrounding stroma, create a proton gradient which provides enough energy to create several molecules of energy-packed ATP. Along with the NADPH produced by the electron transport, the ATP will be used immediately in the biochemical reaction leading to the reduction of CO_2 tot carbohydrate (dark reactions).

The biochemical reaction (CO_2 into carbohydrate)

This light independent reaction occurs in the stroma of the chloroplast. The stroma is a thick, syrupy fluid surrounding the thylakoid membranes. In this reaction CO_2 is bound to a compound known as ribulose 1, 5 bisphosphate**.** When CO_2 enters the cycle, as we can see in Figure 5, a series of steps catalyzed by enzymes takes place. ATP provides the energy for these reactions, while NADH is the reducing agent, attaching hydrogen to form the final product Glyceraldehyde-3P. In this process ADP and $NADP^+$ are been formed. After 3 turns of this circle with help from 18 ATP and 12 NADH the 3 molecules of CO_2 are transformed into a 3 carbon molecule Glyceraldehyde-3P. Two molecules of Glyceraldehyde-3P can be converted into glucose, a 6 carbon sugar and a molecule with great importance for life.

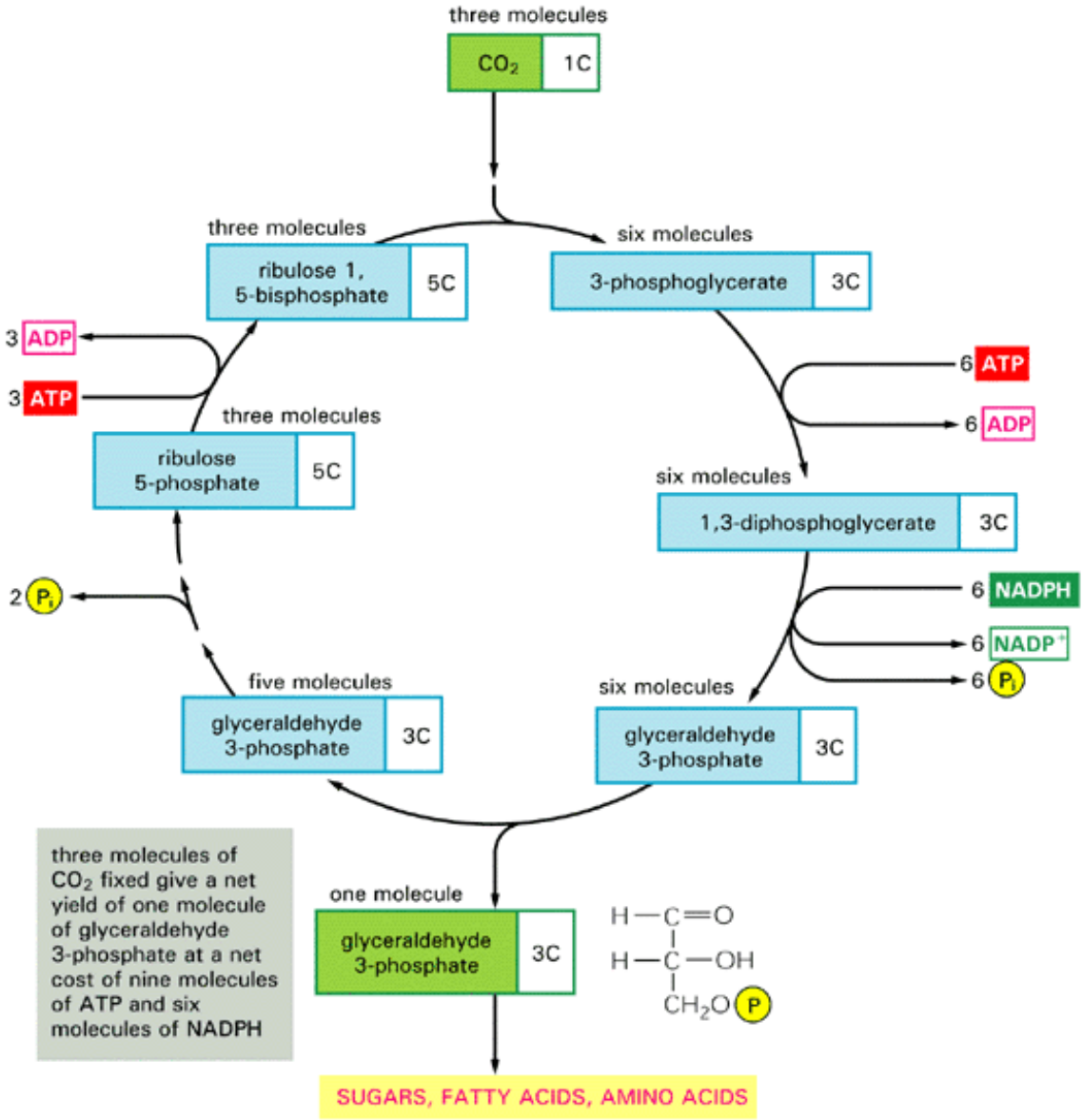

Figure 5. Calvin's Circle. Two of these "turns" as described above are needed to create a molecule of sugar.

The plant produces sugars and organic acids for storage of energy. As mentioned above first sun energy is turned into chemical energy (ATP and NADPH)) through the photochemical reactions and then the ATP and NADPH are used to reduce CO_2 into sugars and other carbohydrates.

Root exudates

Carbohydrates are stored in several parts of the plant including the root system [8]. Up to 60% of the fixed carbon through photosynthesis can be transferred from the leaves to the roots. The root system can produce and release different types of organic compounds into the soil which include: exudates (sugars, organic acids, phenols, and carboxylic acids), gases (ethylene and CO_2), secretions (polymeric carbohydrates and enzymes), and lysates (dead cell materials) [10].

Exudates in the form of sugars are connections of C, H and O. They are categorized as monosaccharides, disaccharides and polysaccharides depending on the number of their monosaccharide on their molecule. In Figure 6 we can see common monosaccharides like those roots are releasing in the substrate.

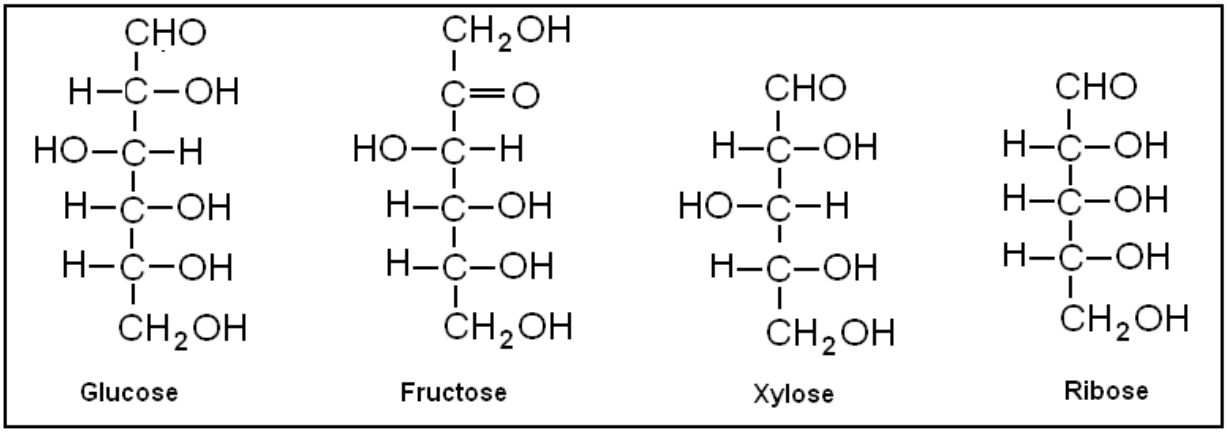

Figure 6. Chemical structure (fisher) of glucose, fructose, xylose and ribose

Exudates as organic acids contain a carboxylic group. Some contain two or contain a ketonic group, the so called ketonic acids. In principal all contain 2-6 molecules of carbon. It can be found at the cytoplasm and the vacuoles of the cells and they have a great role in the metabolism of cells because they are precursors in the creation of carbohydrates, fatty acids or amino acids. Organic acids are involved in many processes operating in the rhizosphere [7].

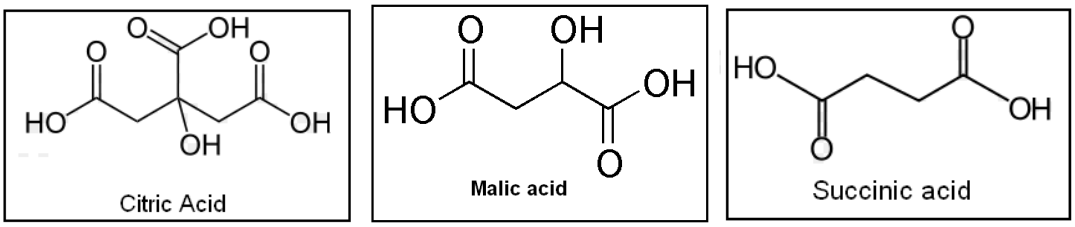

Figure 7. Chemical structure (chain) of some relevant organic acids.

The total of these release processes of the plant roots is called plant rhizodeposition and its products are called rhizodeposits. But how are these rhizodeposits used after release from the plant?

Bacterial symbiosis with plants

Bacteria are microorganisms ubiquitous at every habitat on earth, growing in soil, wastes, seawater and deep in the Earth's crust. They are vitally needed in recycling of nutrients and, in general, many important steps in nutrient cycles depend on bacteria. There are three types of Bacteria based on the kind of energy and the source of carbon they use for growth: the Phototrophic that use sunlight as source of energy, the Lithotrophic that use inorganic compounds and the Organotrophic that use organic compounds, like carbohydrates. Organothrophic bacteria are capable of feeding from glucose (the main type of sugar found in the environment), fructose (found in fruit), sucrose (found in sugar cane), and xylose (found in wood and straw). In other words with the same carbohydrates that plants excrete from their root system previously described as rhizodeposits. This naturally leads into mutually beneficial interactions between plants and micro-organisms. The bacteria can positively interact with plant roots as example forming protective biofilms or by producing antibiotics as biocontrols against potential plant pathogens. Since the largest fractions of rhizodeposits are small molecules they are efficiently synthesized by the plant and efficiently metabolized by bacteria [10]. If bacteria use the rhizodeposits for energy then what are the possibilities for us to use the bacteria for the same reason?

The plant-MFC

In a previous study the plant-MFC, a system capable of producing green electricity by nondestructive harvesting of the rhizodeposits (mainly carbohydrates) of the plant has been presented [10]. The system is based on the principle that the plant rhizodeposits can be utilized as substrates by the bacteria to generate electricity in a microbial fuel cell.

With the microbial fuel cell biodegradable substrates from wastewater or (energy) crops into electricity [2]. The electrochemically active microbes (bacteria) in the MFC act as a kind of biocatalysts using a part of the chemical energy of the substrate for their own metabolism and simultaneously delivering electrons to the anode of the electrochemical fuel cell. The micro-organisms (bacteria) use the anode electrode as the preferred final electrode acceptor because the difference in free energy is larger than other available acceptors such as sulphate [3]. Figure 8 it is presented a model of the plant-MFC.

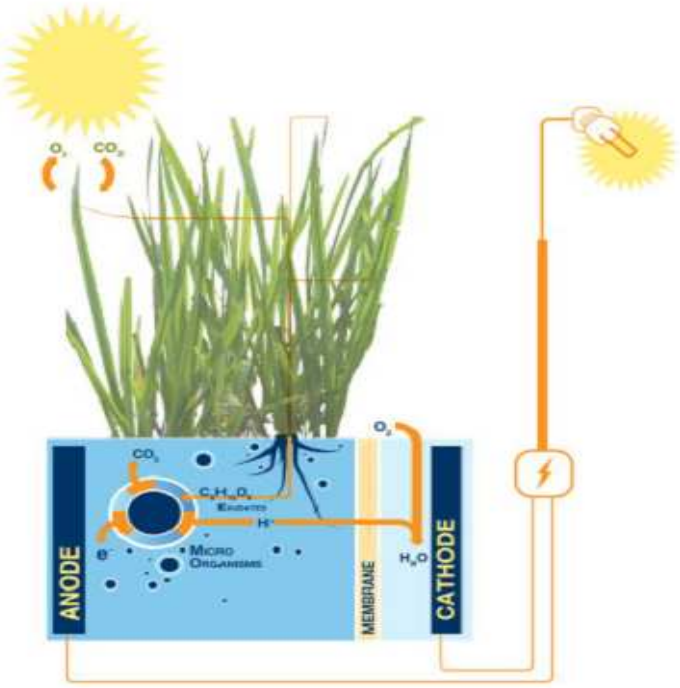

Figure 8. Model of a plant microbial fuel cell producing electricity and driving a light source. Carbon dioxide is fixed and released as rhizodeposits (e.g. root exudates) by the plants and are utilized by micro-organisms that return the carbon dioxide into the atmosphere. The micro-organisms use the anode as electron acceptor for gaining metabolic energy. These electrons flow due to the potential difference from the anode through an electrical circuit with a load or a resistor to the cathode. Hence, electricity is generated which can be used, for example, driving a light source. To remain electroneutrality, protons are transported through the membrane into the cathode where oxygen is reduced with the protons and electrons to form water.

The principals and aim of study

The Plant-MFC is based on two principle ideas, first the energy provided by sun can be stored into the root system of the plants through photosynthesis and second that bacteria can convert the rhizodeposits from plants into usable electrical energy via the microbial fuel cell. To make the Plant-MFC a viable technique, the efficiency of the processes needs to be optimised. In other studies it has been found that rhizodeposition can be stimulated by nutrient limitation, notably iron and phosphate.

The aim of this study is to investigate if limitation of iron and phosphate can improve exudation and the maintain photosynthetic rate of tomato and reed mannagrass plants that are grown under anaerobic root conditions.

Materials and Methods

Plant growth conditions and treatments

Eight plants of reed mannagrass (Glyceria maxima) and four plants of tomato (Lycopersicum esculentum) were allowed to grow in a controlled environment in specially constructed closed boxes made of polyethylene.

The experiment started 1 month after the plantation. A deficiency on phosphorus started 3 weeks after the start of the experiment in order to increase the exudation rate as mentioned on literature [1, 4, 5]. All tomato plants were had their flowers removed to prevent fruit development to keep their energy for growth and storage instead. The root system was grown anaerobically in the box; therefore special seal material was placed on the surface of the box to securely separate the root system from the atmospheric air.

Stonewool was used as substrate with rich given nutrient solution that was allowed to pass to the box through another container (fig.9). The maximum volume of the nutrient solution was 5 litres. The temperature was between 18 and 23^{o}C during day and 15 to 17^{o}C during night. The pH was 6 to 6.5 and the relative humidity 70-75%.

Reed mannagrass was chosen because it is one of the few local species that can efficiently grow in anaerobic riverbank sediments. These anaerobic conditions are necessary for a well-functioning anode compartment of an MFC [10]. Tomato was chosen because it is considered a plant that exudates sugars and organic acids in high amounts.

Root exudates collection

For the collection of the liquid sample were used syringes of 10 ml attached to needles of 12cm that could reach the lowest levels of the box. The syringes were attached to filters to make the samples ready to be used for subsequent HPLC measurements. After collection the samples were stored at -18^{o}C in fridge. The frozen conditions were applied to prevent bacterial activity from metabolizing the exudates inside the vials. To determine possible effects of bacterial metabolism (contamination from root zone) on the amount of sugars and organic acids after restoring the samples from -18^{o}C, we investigated the effects of mixing our samples with standard solutions of glucose and citric acid of known concentrations (see APPENDIX).

As mentioned before, the root system of plants was in anaerobic condition therefore the sample was taken through specially placed sealing membranes on the boxes as we can see in Figure 9.

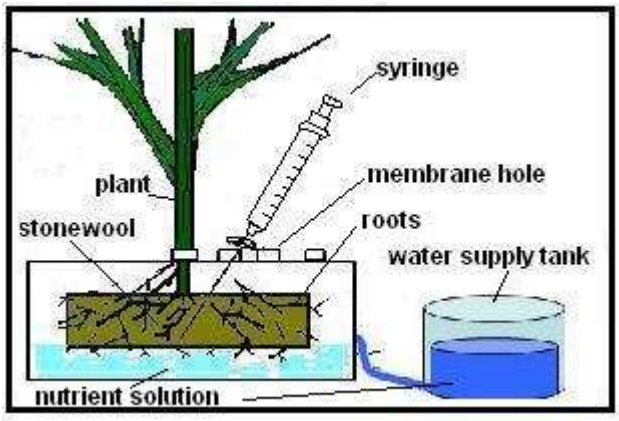

Figure 9.Illustration of exudates sample collection through membrane.

Every week for the total of six weeks a collection of exudates samples was scheduled to be taken on the rhizosphere of the plants. The three first collections were before the phosphorus limitation and the other three after the limitation.

Photosynthesis measurements

The photosynthesis measurements were carried with the ADC LCpro+ portable photosynthesis system. In the LCPro+ device, air is passing the leaf chamber with a constant flow rate. The leaf alters the composition of the air by photosynthesis, respiration and transpiration. An infrared gas analyzer measures the CO_2 and H_2O concentration of the incoming air and in the outgoing air. The photosynthesis and transpiration are calculated and expressed in $\mu mol/m^2/s$.

For the measurements the attached chamber was used and a pre-set illumination sequence was selected in the software. Attached leaves of tomato plants were measured at 20 cm and 70 cm from the base and the results were calculated in average for these two measurements.

Figure 10.Picture of ADC LCPro+ leaf chamber while measuring leaves of tomato plant.

*It has to be noted here that LCpro+ had technical problems and although it was planned a weekly measurement of photosynthesis it was limited only for begin and end of the experiment.

Preparation of samples for HPLC analysis

Eight samples of reed mannagrass and four samples of tomato were prepared for the HPLC analysis with the help of accurate pipette (1 ml).
Seven standards solutions were prepared for comparing the exudates samples with the known concentrations. The standard solutions were prepared based on reported exudates in previous studies [1]. The.sugars were: fructose, glucose, ribose and xylose and the organic acids: citric, succinic and malic. A 1 ml sample was prepared for each standard with known concentration of 500mg/L (0.05%).

Quantitative determination of sugars and organic acids

The quantification of the organic acids and sugars was carried with a Shodex RI-71 series high performance liquid chromatography (HPLC) system. Organic acids were separated using a column suitable for organic acid and sugars analysis. The mobile phase was 1.25 mM H_2SO_4 at a flow rate of 0.4 ml/min. The wavelength of UV detector was set at 512 nm. The temperature of the water bath containing the reaction coil was at 70 ºC and the reaction time was approximately 1 min.

The identification of exudates was made by comparing the retention time (min) of the standard solutions and the retention time of the exudates samples.

The determination of quantity was made comparing the height (Height mV) and the area covered (area mV per*min) between the exudates samples and the standard solutions as appeared after the detection. At the start of the experiment all standard solutions were tested in known concentrations for the calculation of sugars and organic acids concentration. All standards had a concentration of 0.05%.

Organic material oxidization (COD)

The determination of organic material was carried with the COD method (chemical oxygen demand). Organic material is oxidized by potassium dichromate in acidic conditions and a catalyst (Ag^+).By adding of Hg^{2+} the catalyst is protected from sedimentation with Cl^- .The reduced quantity of chromate can be determined photometric and is related to the COD of the sample .

Totally 20 samples were prepared for COD calculation. Aim was to detect the alteration of organic material inside the boxes where the plants grow. Samples were chosen from the second week, the fourth week and the sixth week. Two samples were collected from the nutrient solutions to be tested as standards (a complete nutrient solution sample and a sample without phosphorus).

Results

Plant condition

On the second week of our experiment symptoms of purple coloration on the leaves was observed. After the third week bursting of the epidermis was noticed. Although the root system was examined at the end of the experiment and its condition seemed to be good, the tomato plants seemed to have difficulties to adjust. At the fourth week one of the plants suffered from dryness and started to lose color when after the fifth week our tomato plants started to have serious problems of anoxia and growth stop. The symptoms are presented at the APPENDIX (Figure 36).

Photosynthesis results

The photosynthesis and transpiration measurements show a reduction from the start of the experiment to the end of the 6 week period.

The first measurements taken on the leaves show a photosynthetic response starting from 0 and gradually advances to 25 µmol/m^2/s for all plants in average as we can see below (Fig 11). The transpiration starts from 2 and reaches 4 µmol/m^2/s at the maximum light intensity of 1000 µmol PAR/m^2/s (Fig 12) again for all plants in average.

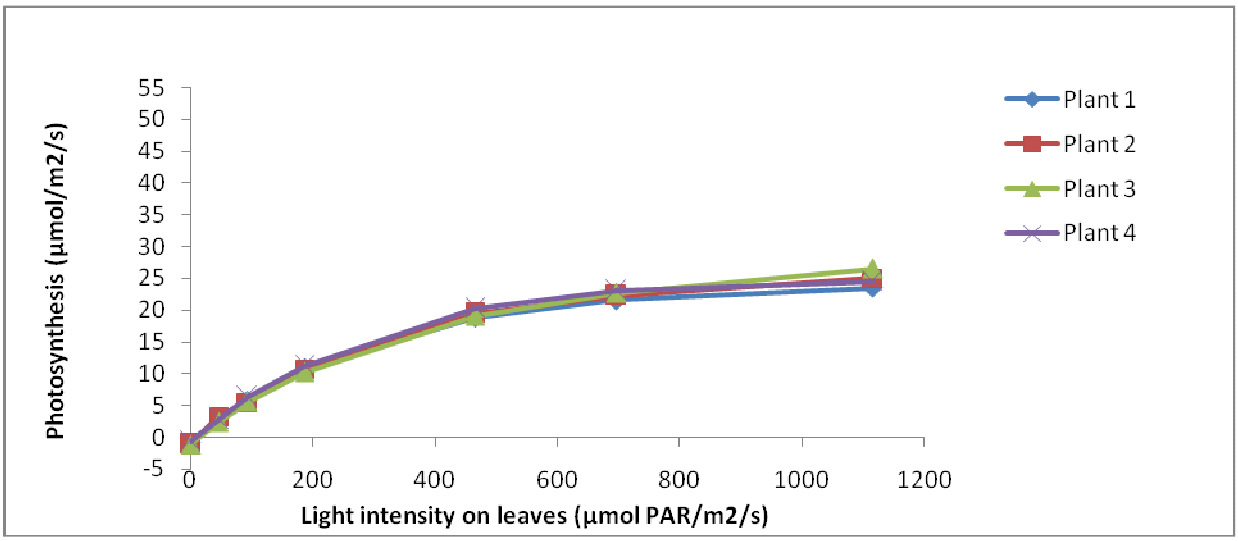

Fig 11. Effects of light intensity to the photosynthesis of tomato leaves at the begin of experiment

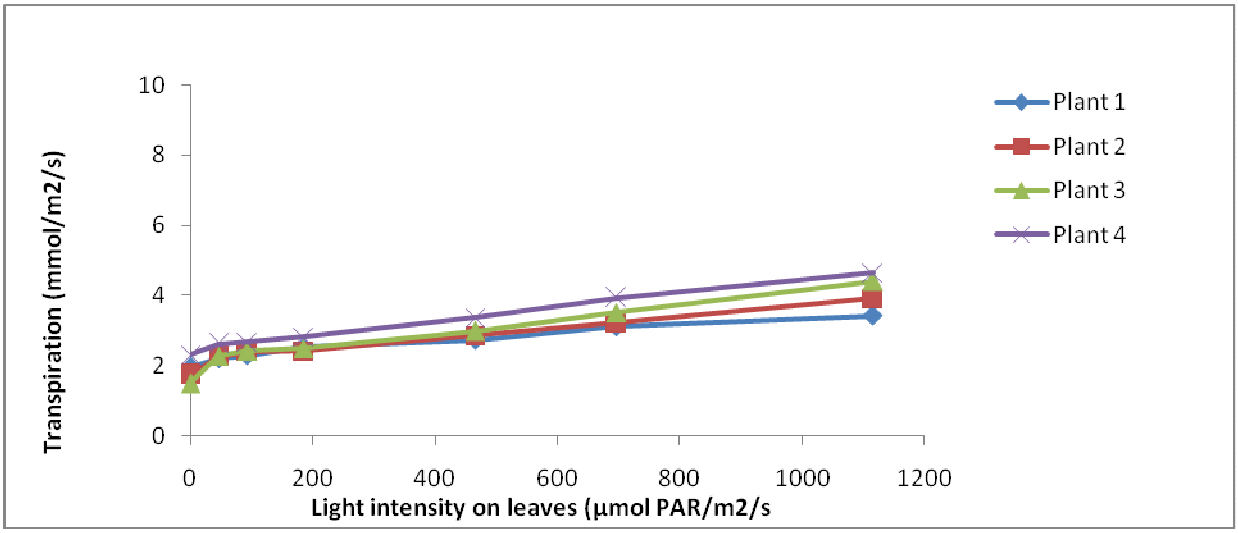

Fig 12. Effects of light intensity to the transpiration of tomato leaves at the begin of experiment

The measurements taken the sixth week on the leaves of the first and the second plant show a photosynthetic response that start from 0 and gradually advances to 17 and 20 µmol/m^2/s as the light intensity reaches 1200 PAR (Fig 13). Tomato plant 4 shows a maximum level of 10 µmol/m^2/s at 500 PAR and then a decrease follows. Plant 3 was constantly at 3 µmol/m^2/s in average until the end of the measurements. The transpiration on plants 1 and 2 starts from 1 µmol/m^2/s and reaches almost 2 in average for both plants. Plant 4 again shows some transpiration at a level of 1 µmol/m^2/s at all light intensities but plant 3 reacts very weak to light at a rate below 0,5 µmol/m^2/s. Plants 3 and 4 show very weak performance in both levels at the sixth week. As mentioned above the photosynthesis and transpiration levels were lower than the first measurements

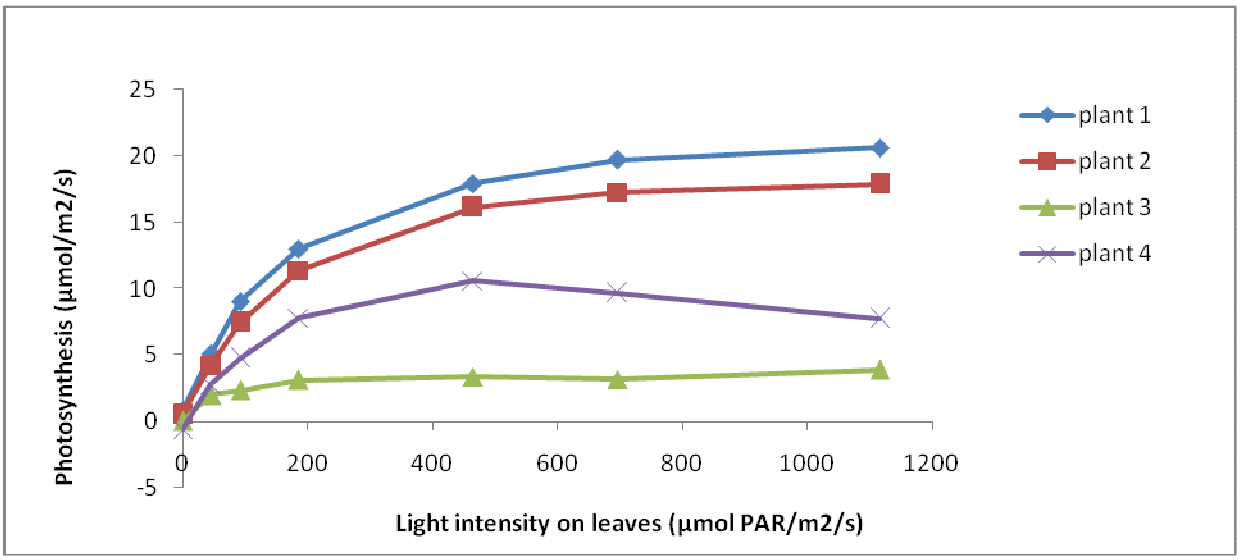

Fig 13. Effects of light intensity to the photosynthesis of tomato leaves at the end of experiment

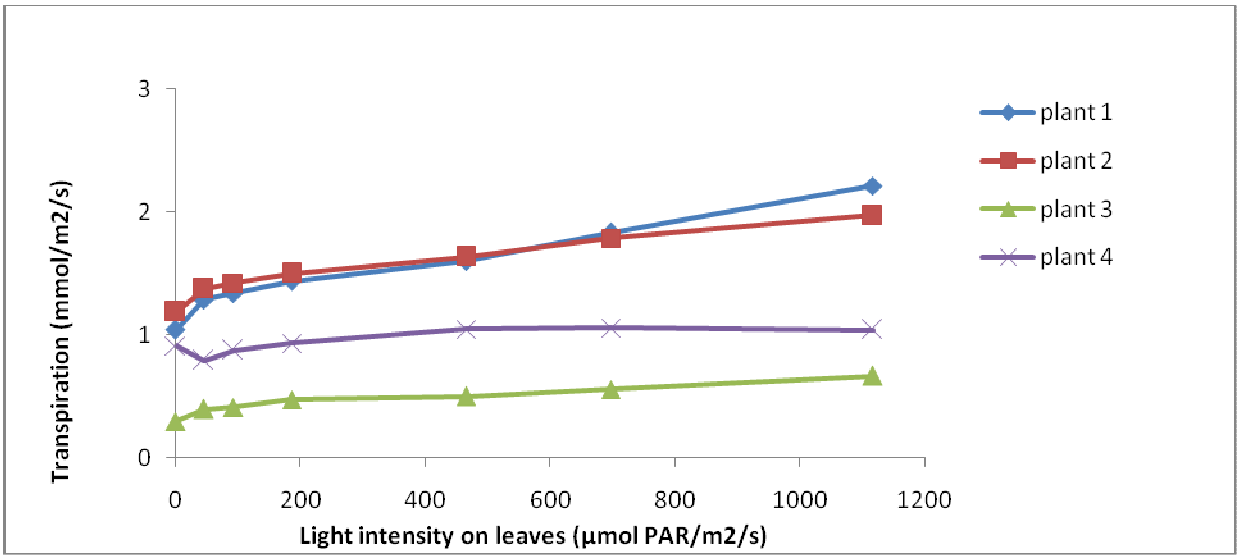

Fig 14.Effects of light intensity to the transpiration of tomato leaves at the end of experiment

Exudation results

The exudation results show no exudates on our samples for the total period of the experiment.

As explained in the Materials & methods the standard solutions where analyzed of known concentrations to compare the Height mV and Area mV*min with our samples. After the analysis on the HPLC detector, every standard sample revealed a "peak" that could be identified at certain time. For the fructose the peak reached 26,211mV and covered an area of 33,986 mV*min which equals to 0.05% concentration. We can see all standard solutions results below (Table 1).

Standard solution	Height (mV)	Area (mV*min)	Concentration (%)	Retention time(min)
Fructose	36,211	33,986	0.05	12.000
Glucose	36,062	32,331	0.05	11.300
Xylose	40,840	44,442	0.05	13.700
Ribose	36,897	43,567	0.05	13.500
Citric acid	20,789	30,390	0.05	9.150
Succinic acid	25,367	31,021	0.05	13.700
Malic acid	28,797	31,670	0.05	11.200

Table 1. Height mV and Area mV*min of standard solutions with known concentration 0.05%.

In our samples collected at the first week there were no peaks that could be identificied as sugar or organic acid. There were no peaks matching the retention time of any of the used standards. This holds for the total period of the experiment. The concentration of sugars or organic acids based on the match of retention time between standards and exudates samples was below our detection limit. The analysis (graphic form) directly from the HPLC of the exudates samples is been shown on the APPENDIX. We can see that there was no "peak" identified in our samples that could match any of the standards peak.

		Average Concentration(%) per plant						
Week	**Plant sample**	**Fructose**	**Glucose**	**Xylose**	**Ribose**	**Citric acid**	**Succinic acid**	**Malic acid**
1	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
3	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
4	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
5	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
6	Tomato	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	Reed Mannagrass	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 2. Alteration of Sugars and organic acid composition on average in tomato and reed mannagrass per week. n.d.: not detectable.

Organic material results (COD)

The COD method showed that there was no increase in the organic material that could be oxidized in our exudate samples.

Obviously there was already organic material in the nutrient solution which revealed 189 mg/L in the standard solution and 190 mg/L for the -P solution. Compared with the results of the exudates samples we can see that the amount of organic material in the nutrient solutions was higher .Tomato showed 176 mg/L the second week in average, 145 mg/L the fourth week after the phosphorus deficiency and 158 mg/L on the sixth week at the end of the experiment. Reed mannagrass showed 156 mg/L the second week then a little higher oxidized material in the fourth week's samples after the deficiency and 143 mg/L at the end of the experiment. All samples were reduced in oxidized material compared to the Nutrient solution and –P.

Week	**Sample**	**Average oxidized organic material(mg/L)**
-	Nutrient solution	189
	Nut. Solution -P	190
2(before deficiency)	Tomato	176
	Reed mannagrass	156
4(after deficiency)	Tomato	145
	Reed mannagrass	162
6(after deficiency)	Tomato	158
	Reed mannagrass	143

Table 3. Alteration of average oxidized organic material in between week two, four and six.

Summary & Discussion

On photosynthesis

The aim was to determine and compare the photosynthetic rate in leaves of tomato plants that were grown under control and nutrient limited conditions.

Our results show that the photosynthetic rating as well as the transpiration of the leaves has decreased during the experiment if compared to the first measurements.

We can see that in the first measurement of photosynthesis and transpiration all plants are having the same response. In the last measurement we can see that plant 1 and 2 have similar response curve. At this point it has to be mentioned that plant 3 had difficulties of surviving from the fifth week onwards and therefore the photosynthesis may have been affected by its condition. Plant 4 also had difficulty gaining enough water and nutrients since the storage container that was providing the nutrient solution did not function well. That is probably why we can see a lower photosynthetic activity and transpiration on plant 4 in week 6. Besides the technical problems in plant 3 and 4 we can see that all plants suffer from low photosynthetic response compared to the start of the experiment.

Although factors like chlorophyll concentration on the leaves, age of the plant or stomata state can affect the photosynthetic rate, it looks more reasonable to assume that the concentration of the sugars in the leaves affected the results (besides plant 4, which was also suffering from low water supply and plant 3 that was nearly dried out at the time of the measurement). It is known that high concentration of sugars in the leaf can inhibit photosynthesis. All the flowers of the plant were removed to minimise loss of sugars to the fruit. The phenotype of the plant indicates large amounts of sugar in the leaves (see Fig.36). The fact that flower removal leads to an increase in leaf sugar content indicates that these sugars are not very efficiently transferred to the roots for exudation.

Another reason that could lead to the decrease of photosynthesis is the anaerobic condition in the root zone. Although anaerobic conditions are necessary for a well-functioning anode compartment of an MFC, they are not a suitable environment for the tomato plant. Maybe stressfull conditions lead to more exudation but what if the stressfull state of the plant also lowers the photosynthesis of the plant (and the fixed carbon)? In that situation the Plant exudation could be less productive in total.

On root exudation

In this study the aim was to determine the concentration of sugars and organic acids in our samples taken from tomato and reed mannagrass plants over the experimental period of six weeks. As shown in the section Results, there is no presence of detectable amounts of glucose, fructose, xylose, ribose, citric acid, malic acid or succinic acid in our samples. These exudates are the main exudates we could expect [1].

Other peaks that were present in our samples, as seen on the HPLC results (see APPENDIX), are not exudates and we cannot say that they have any relation to the

standard samples we used for identification of sugars or organic acids because they are detected in different time. These peaks if we look carefully are present also in the nutrient solution that contain no rhizodeposits. We therefore conclude that these compunds are not exudate material from the plant.

Based on the concentrations of the used standards we conclude that if any of such exudates were present in our samples, then its concentration should be much lower that 0.05%. In previous studies [1] the results on exudates analysis were around 5.85 mg/L for Glucose, 10.53 mg/L for fructose and 93,4 mg/L for citric acid which shows the difference from our results. Another example for succinic acid the mg/L per tomato plant was 61.5mg/L . The volume of water in our boxes was 5 L max. 60/5 is equal to 12mg/L that could be our expected amount. Since we used bigger plants of total weight (215gr of dry weight in average) we could expect values of 60-200 mg/L per plant in case of anaerobic grown root system although younger plants are exudating considerably higher amounts [6].

Another possible reason why we might not have detected any sugars or organic acids could be the bacterial metabolism. Although our related study (see APPENDIX) showed only reduction of sugars in the vials it could also suggest that bacteria reduce the amount of sugars in the substrate to an amount of 0.5% per 3.45 hour. For citric acid we did not find a reduction.

On oxidized organic material

The results show that there was no increasing of the organic material that could be oxidized like sugars or organic acids on our samples.

In addition, the samples reveal that the nutrient solution lost organic material in the process probably because the plant was using the nutrient solution’s compounds. In exchange we would expect increasing of organic material that can be oxidized due to exudates increase but that was just but an expectation.

Conclusions

The tomato plants at the end of the experiment were suffering from low photosynthetic rate and low transpiration, most probably due to accumulation of sugars in the leaves. The plant probably suffered from the anaerobic root environment in combination with nutrient limitation. This might stress the plant for more exudation but might be lowering its photosynthesis and its general condition as well.

As mentioned above, there were no detectable exudates present on our samples. In the case of sugars this absence might be caused by microbial activity in the sample between thawing and measurement. Since the expected values, as for example for succinic acid should be around 60-200 mg/L, there must be a factor that is negatively affecting the rhizodeposition in our plants. This could suggest that there are either internal factors that prevent the rhizodeposition or either external factors that reduce the amount of exudates after rhizodeposition maybe while the sugars or organic acids are accumulating in the substrate.

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APPENDICES

HPLC results of exudates and standard solutions

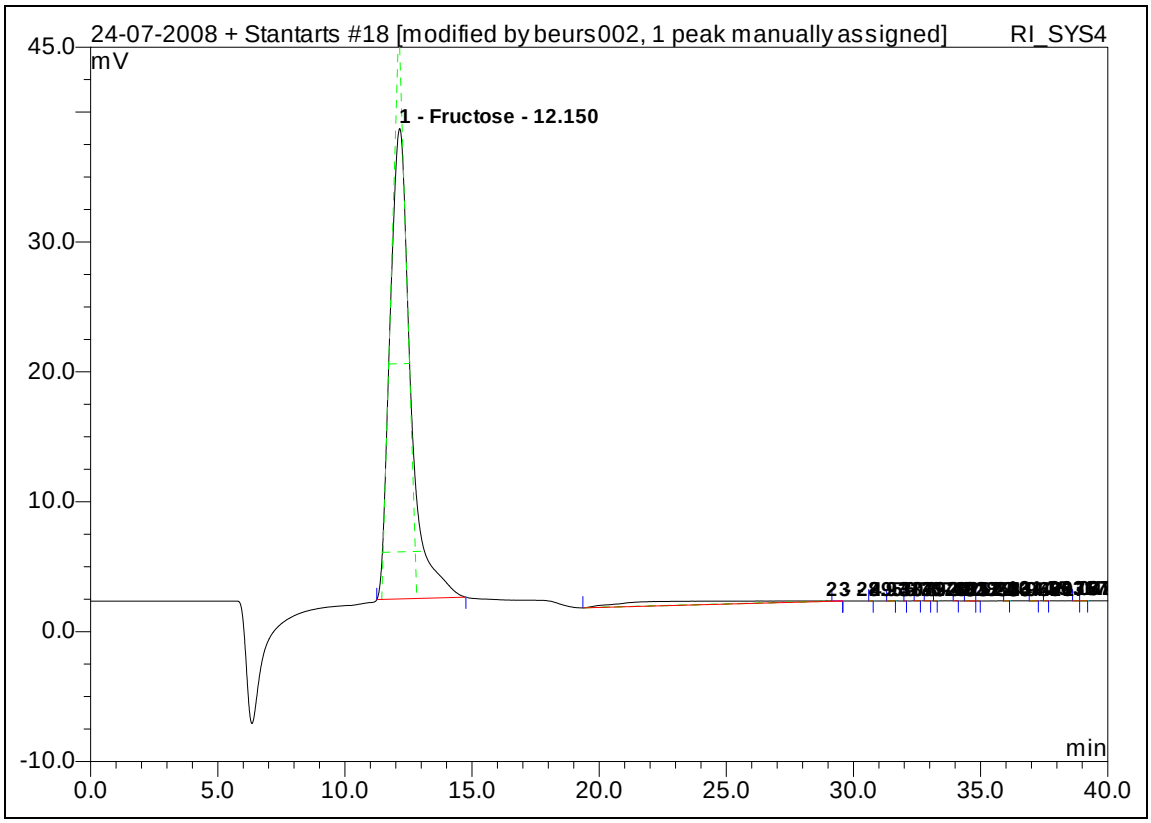

Fig. 15 Peak result of Fructose sample with 0.05% concentration analyzed on HPLC.

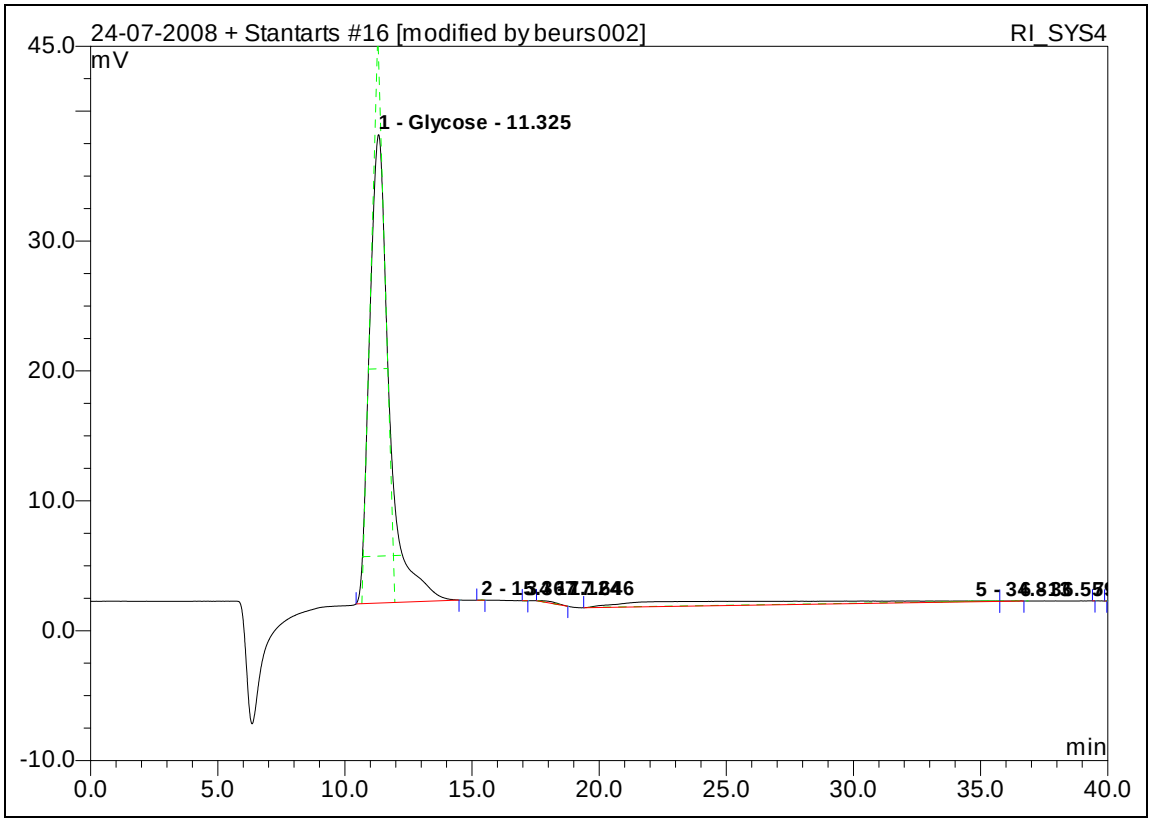

Fig. 16 Peak result of Glucose sample with 0.05% concentration analyzed on HPLC.

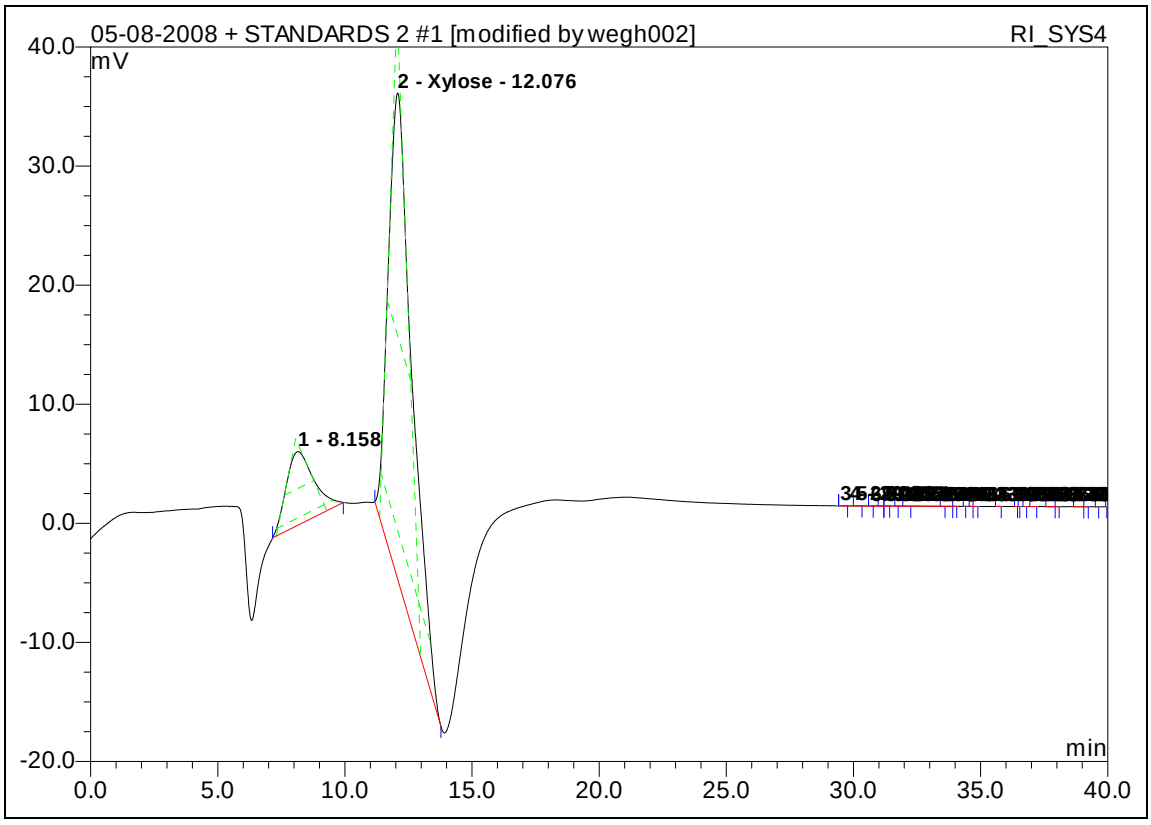

Fig. 17 Peak result of Xylose sample with 0.05% concentration analyzed on HPLC.

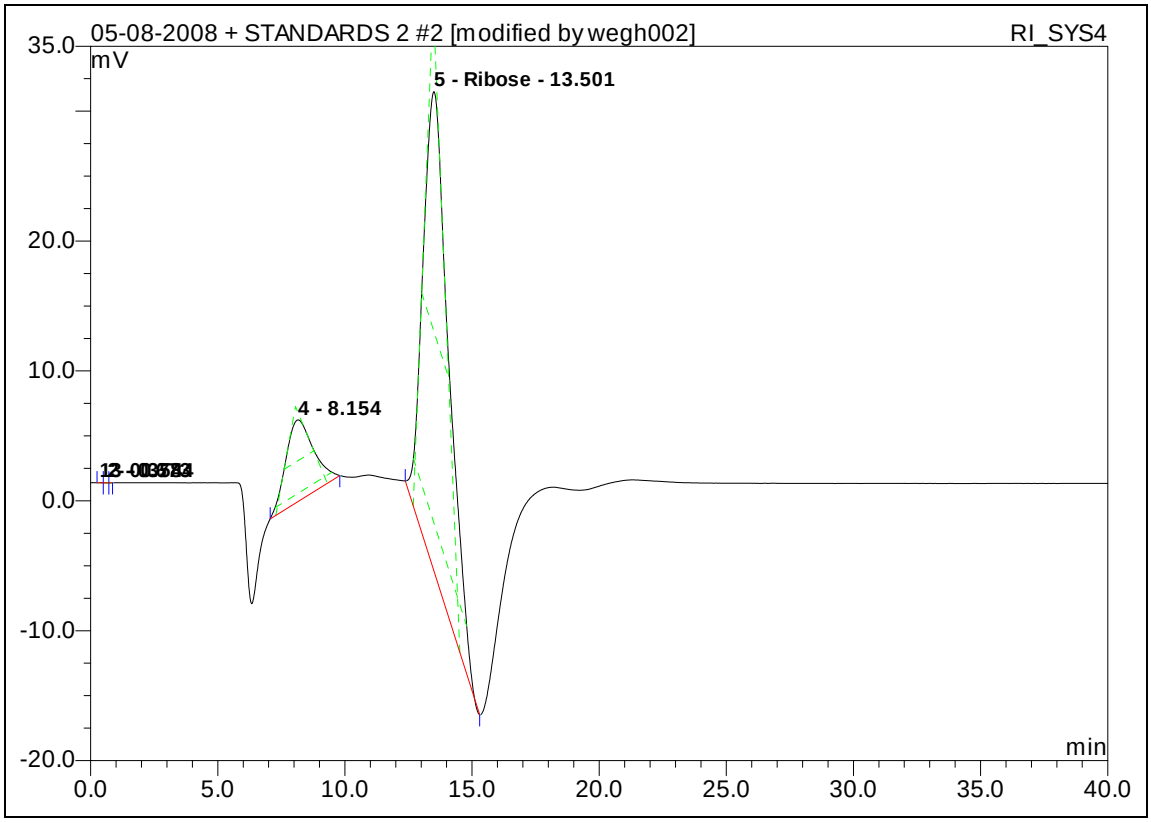

Fig. 18 Peak result of Ribose sample with 0.05% concentration analyzed on HPLC.

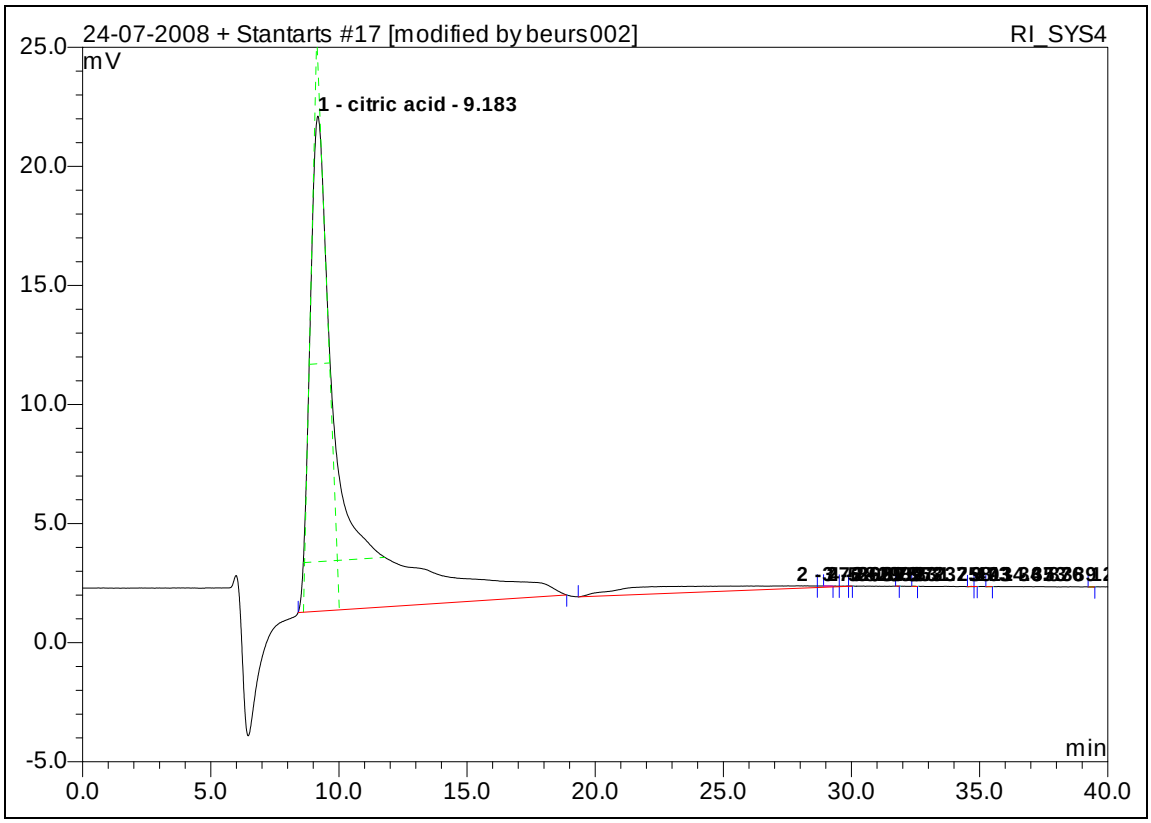

Fig. 19 Peak result of Citric acid's sample with 0.05% concentration analyzed on HPLC.

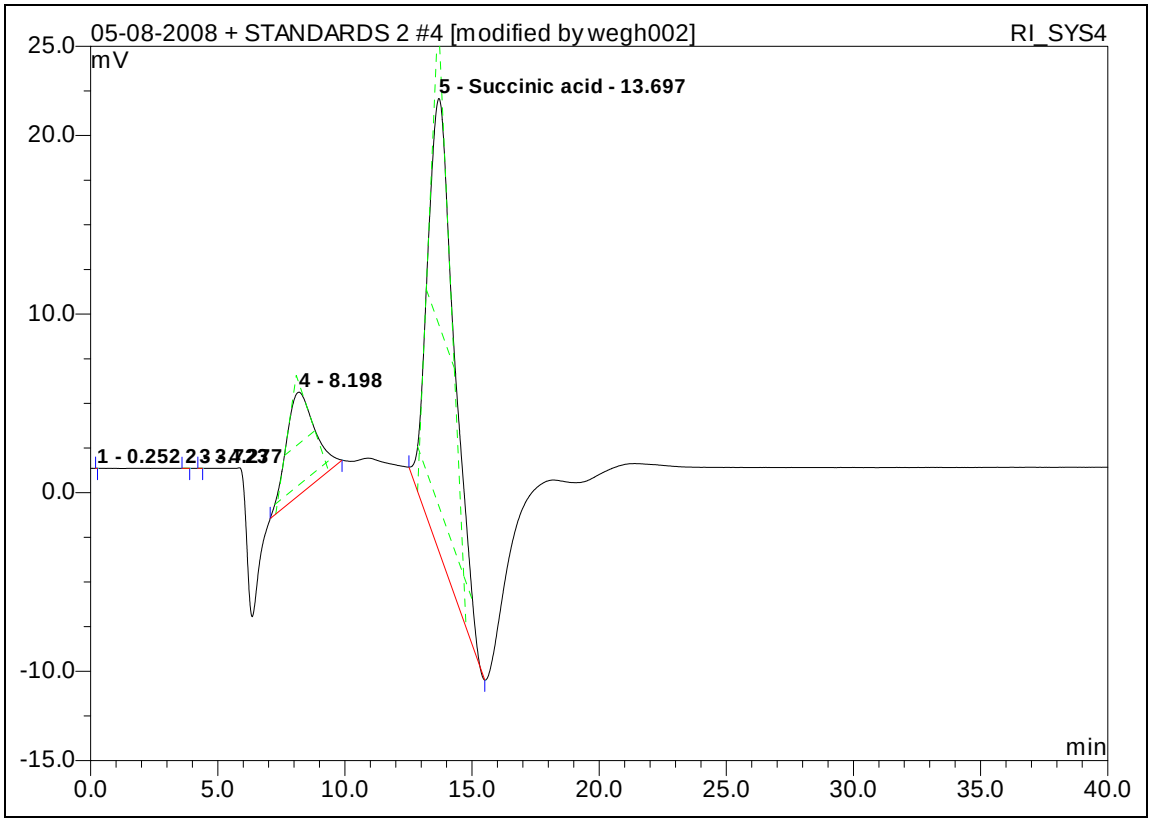

Fig. 20 Peak result of Succinic acid's sample with 0.05% concentration analyzed on HPLC.

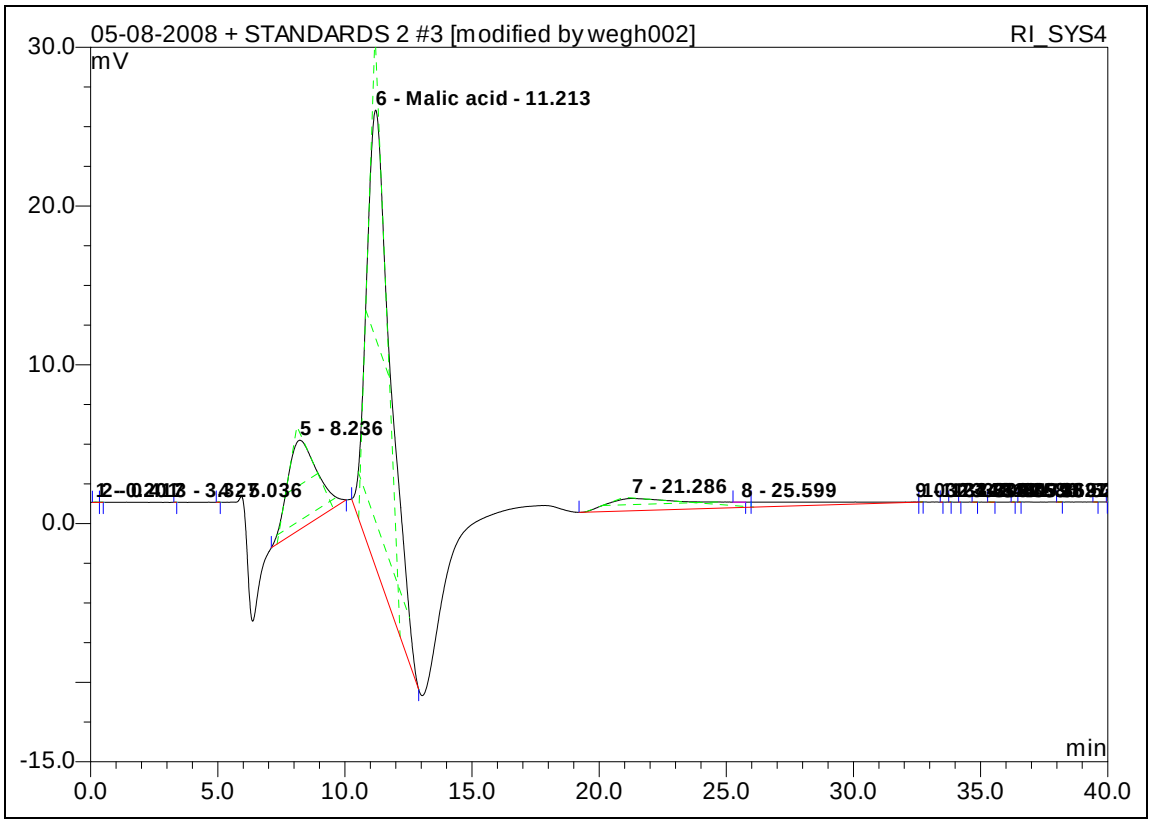

Fig.21 Peak result of Malic acid's sample with 0.05% concentration analyzed on HPLC.

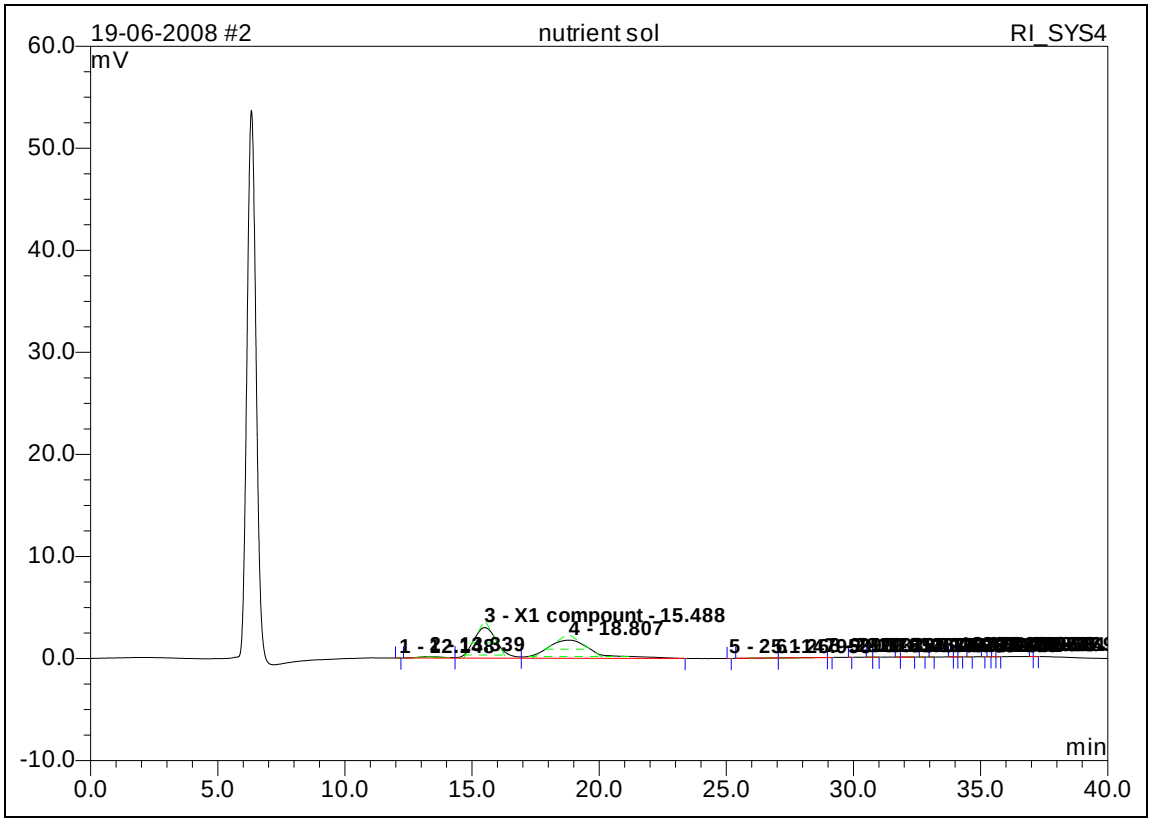

Fig. 22 Peak result of Nutrient solution sample analyzed on HPLC.

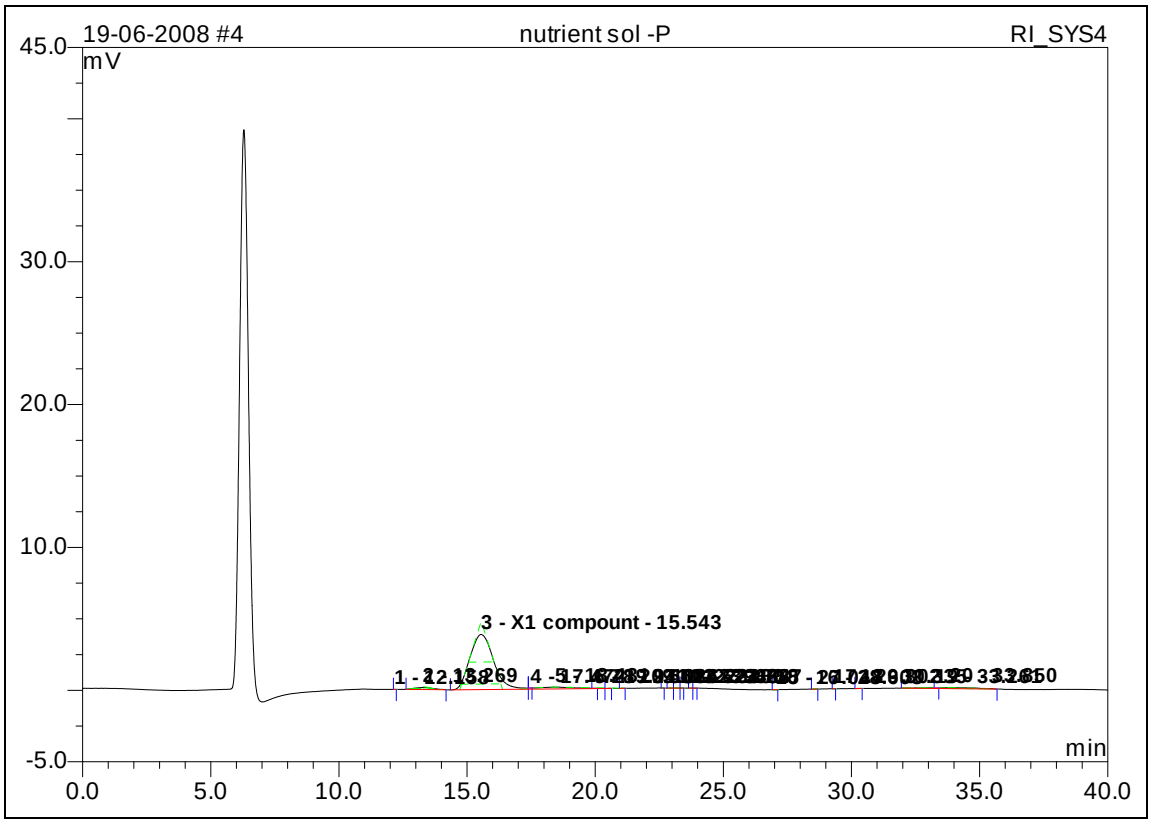

Fig. 23 Peak result of Nutrient solution without phosphorus sample analyzed on HPLC.

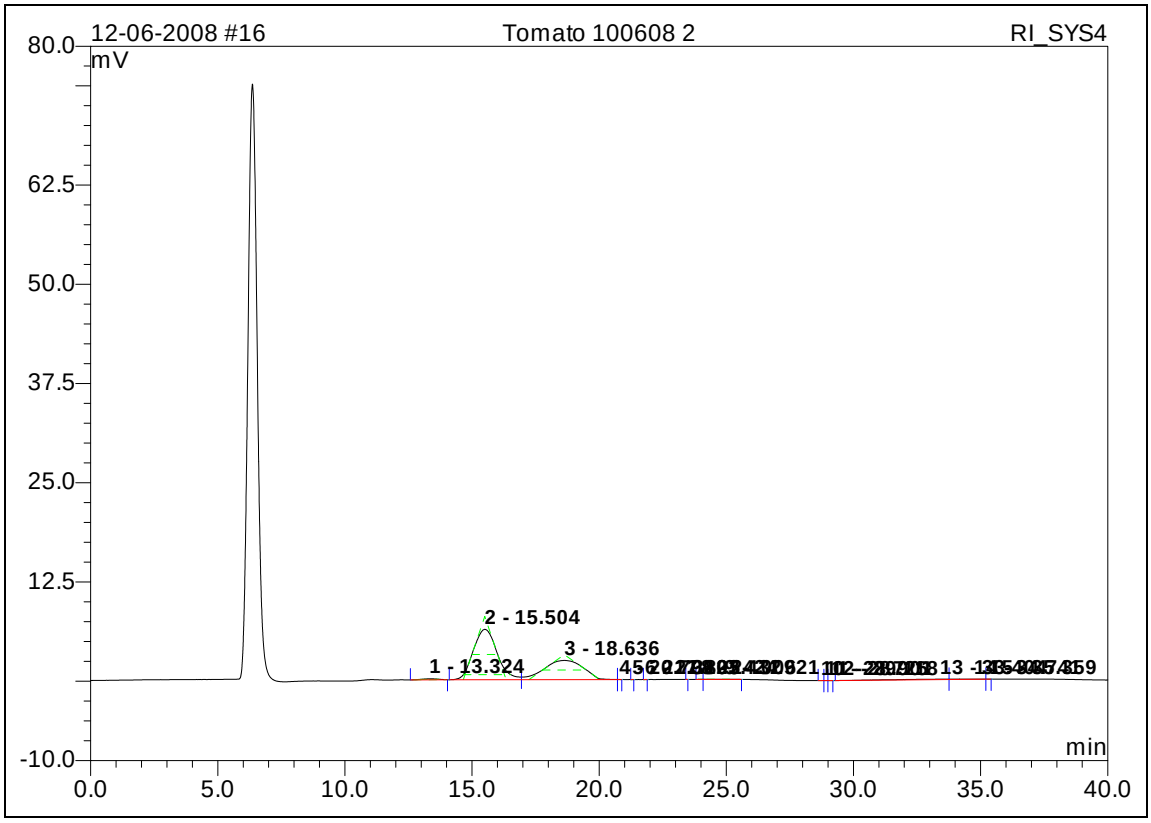

Fig. 24 Peak result of Tomato representative sample from the first week analyzed on HPLC.

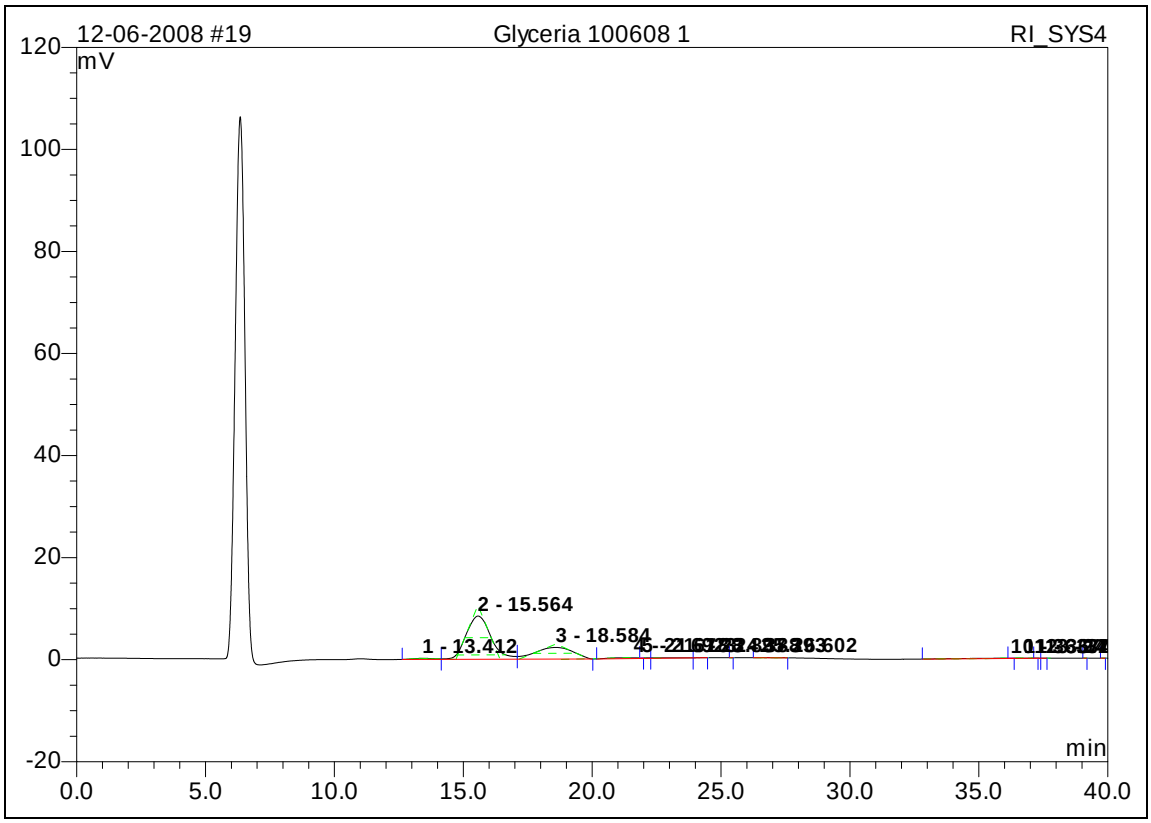

Fig. 25 Peak result of Reed mannagrass representative sample from the first week analyzed on HPLC.

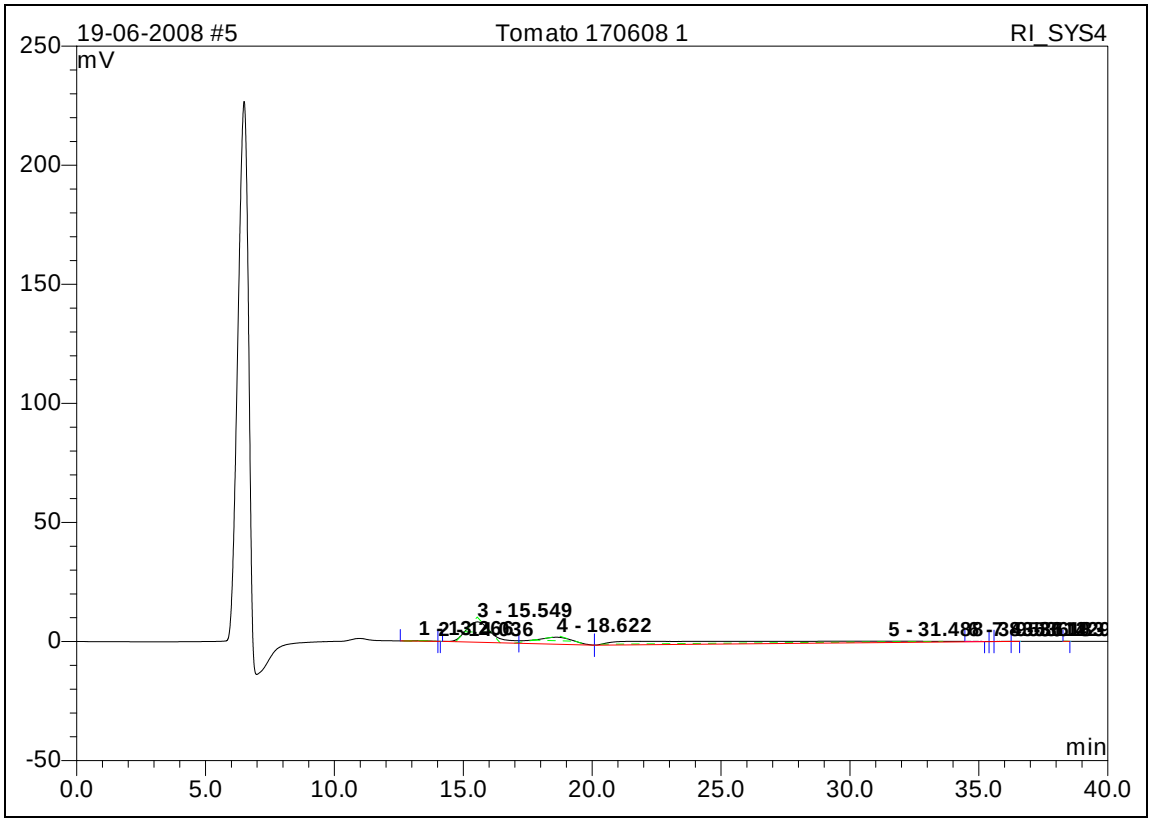

Fig. 26 Peak result of Tomato representative sample from the second week analyzed on HPLC.

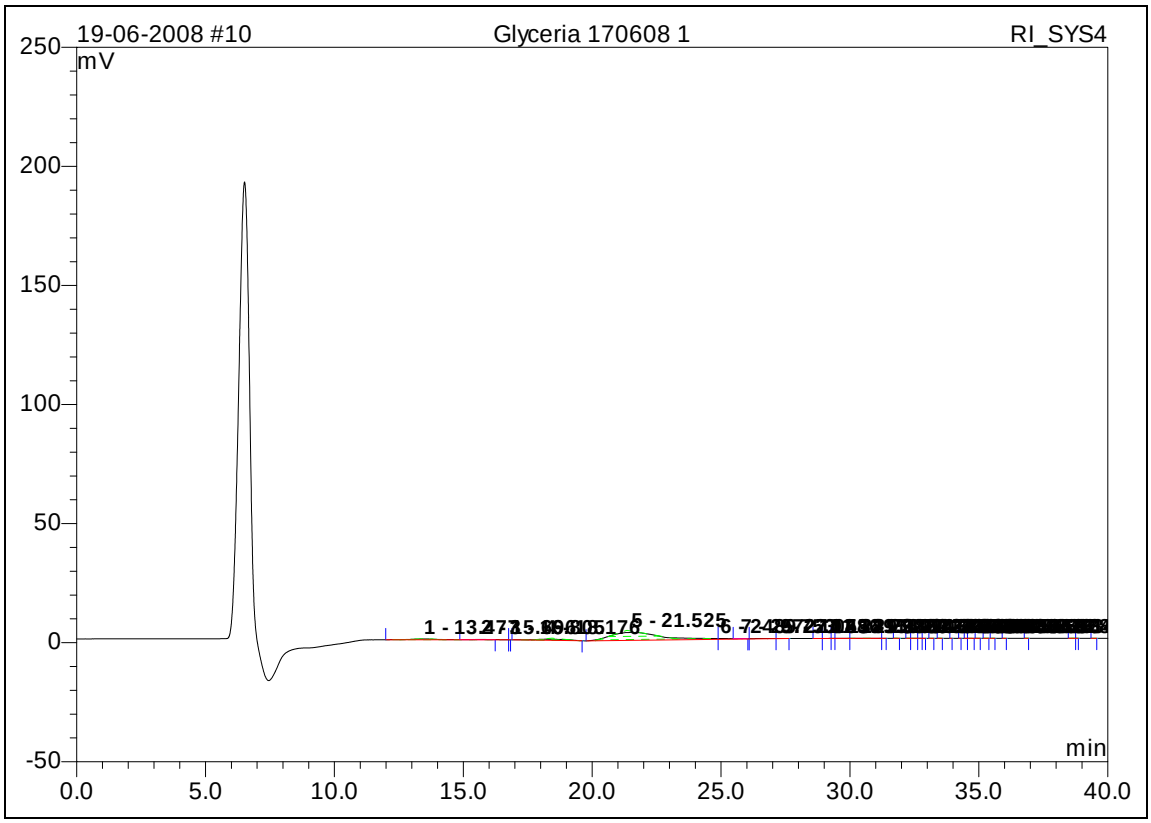

Fig. 27 Peak result of Reed mannagrass representative sample from the second week analyzed on HPLC.

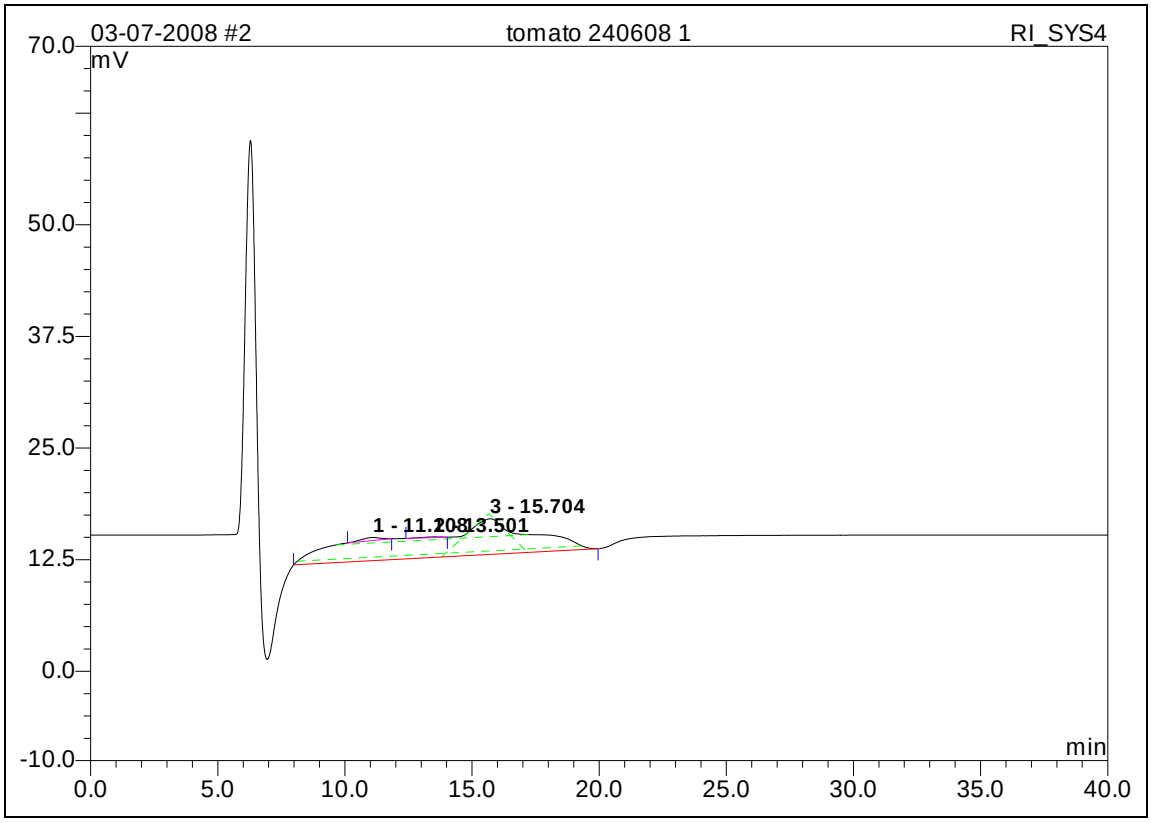

Fig. 28 Peak result of Tomato representative sample from the third week analyzed on HPLC.

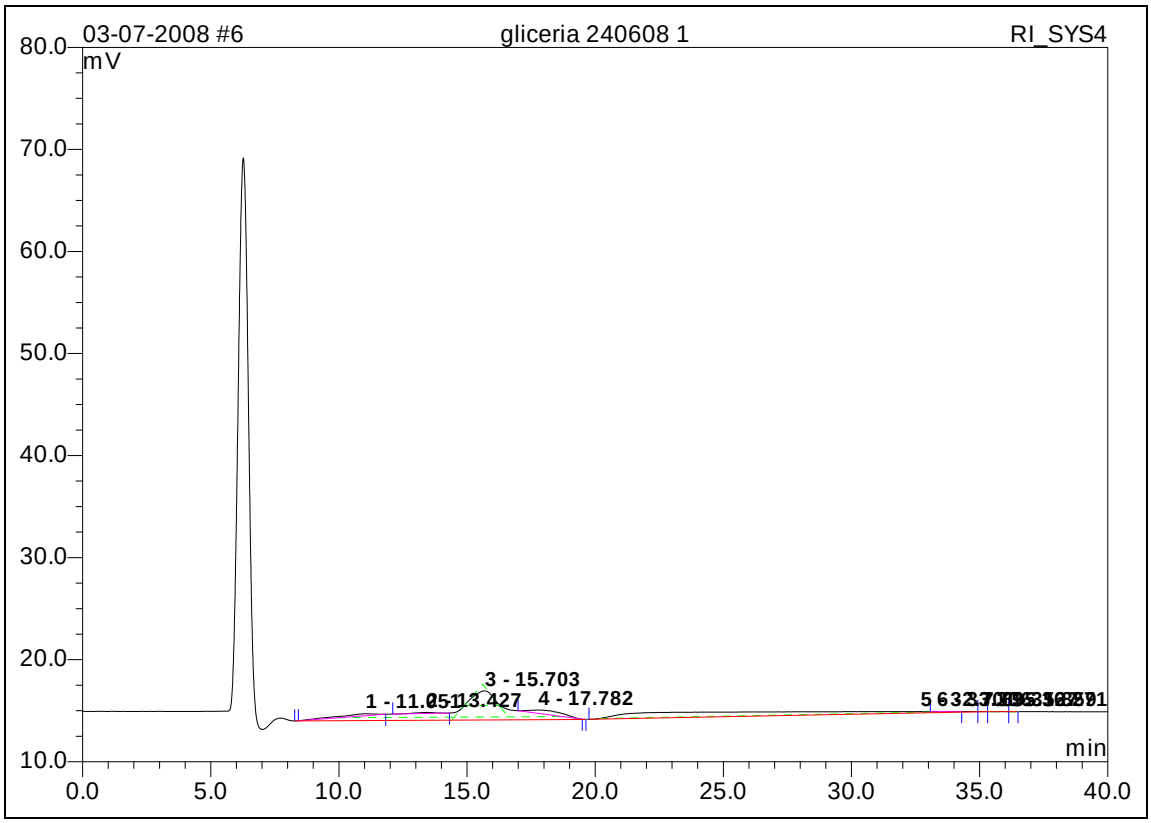

Fig. 29 Peak result of Reed mannagrass representative sample from the third week analyzed on HPLC.

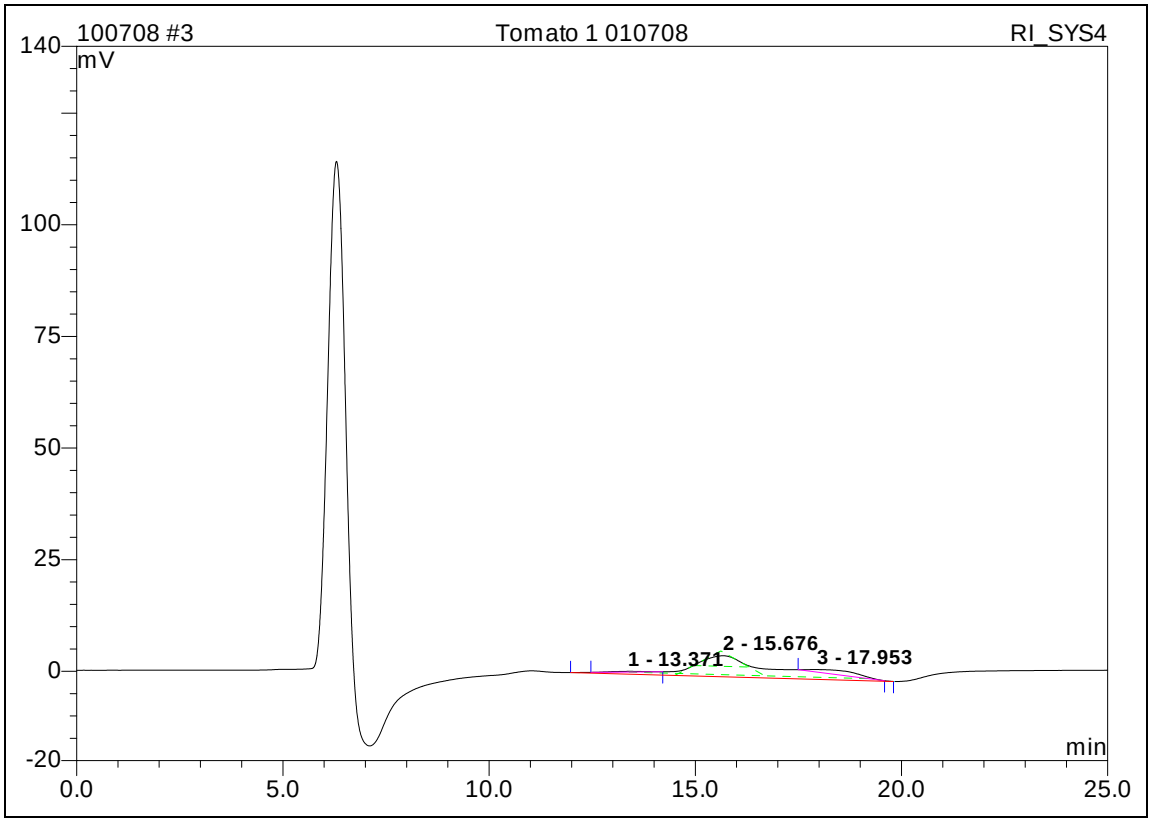

Fig. 30 Peak result of Tomato representative sample from the fourth week analyzed on HPLC.

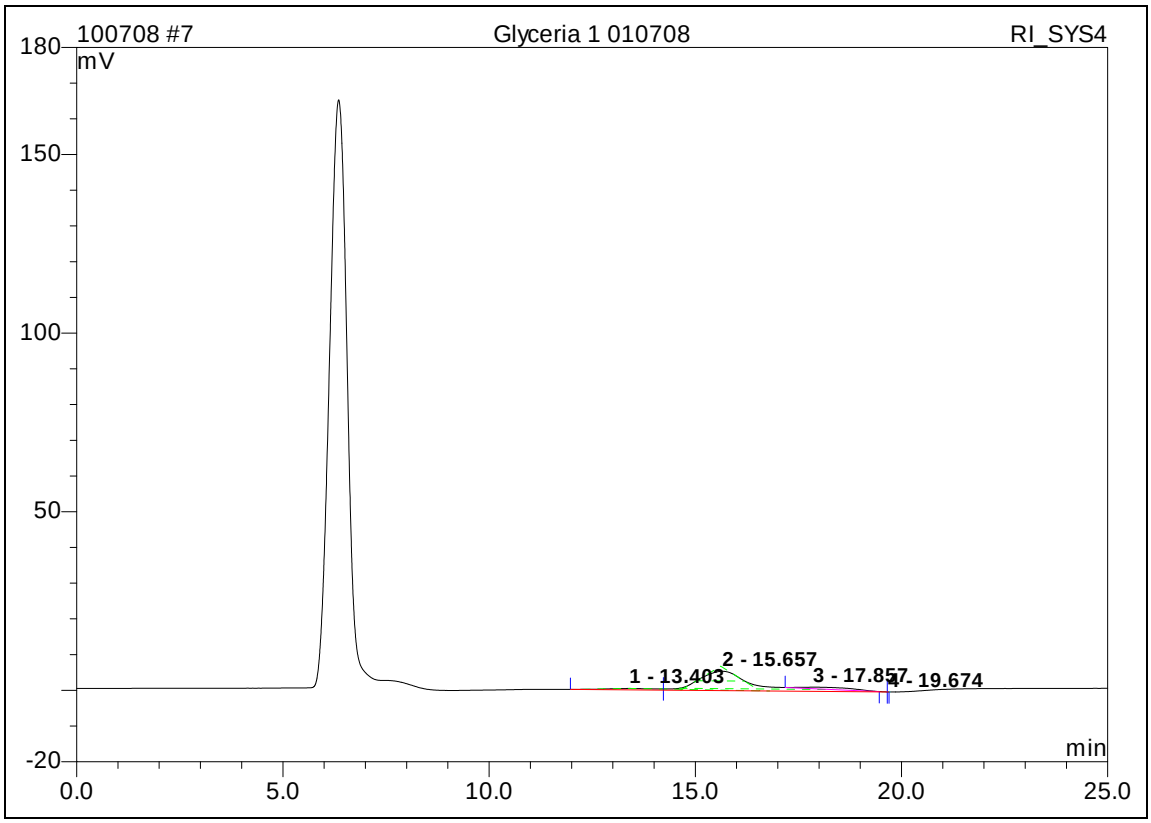

Fig. 31 Peak result of Reed mannagrass representative sample from the fourth week analyzed on HPLC.

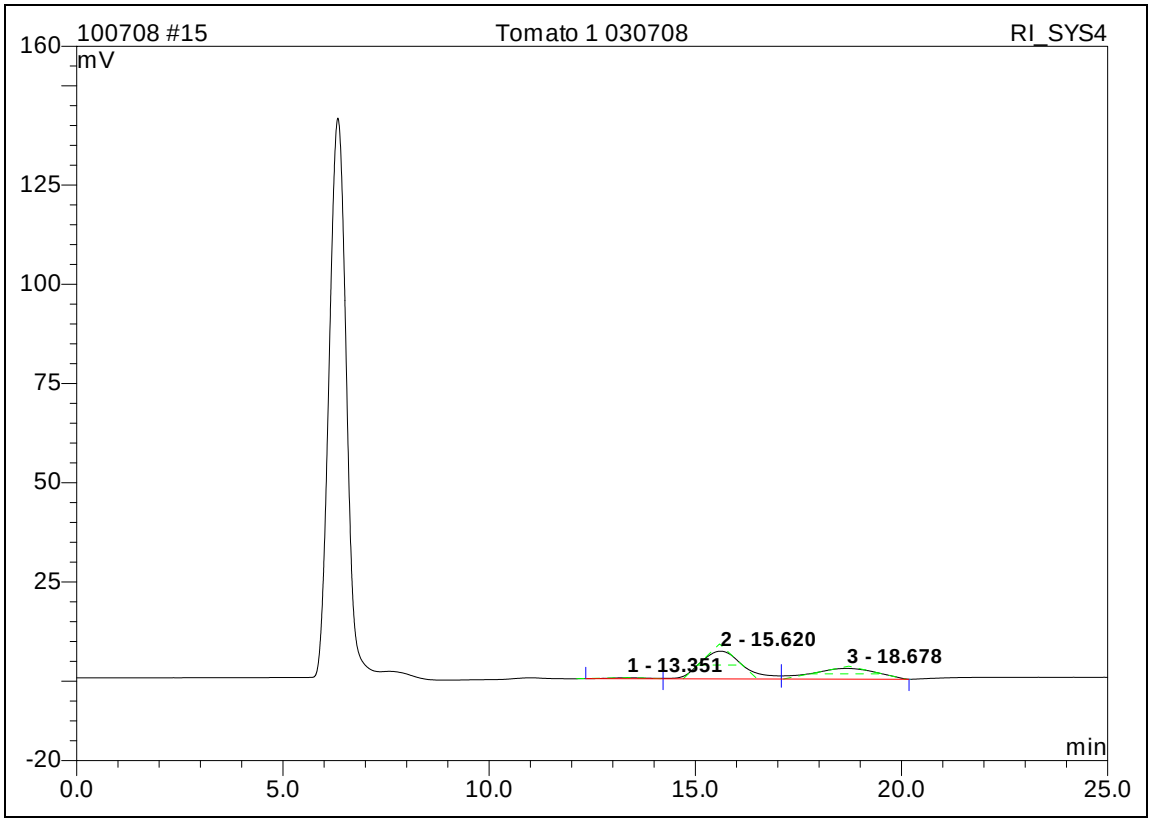

Fig. 32 Peak result of Tomato representative sample from the fifth week analyzed on HPLC.

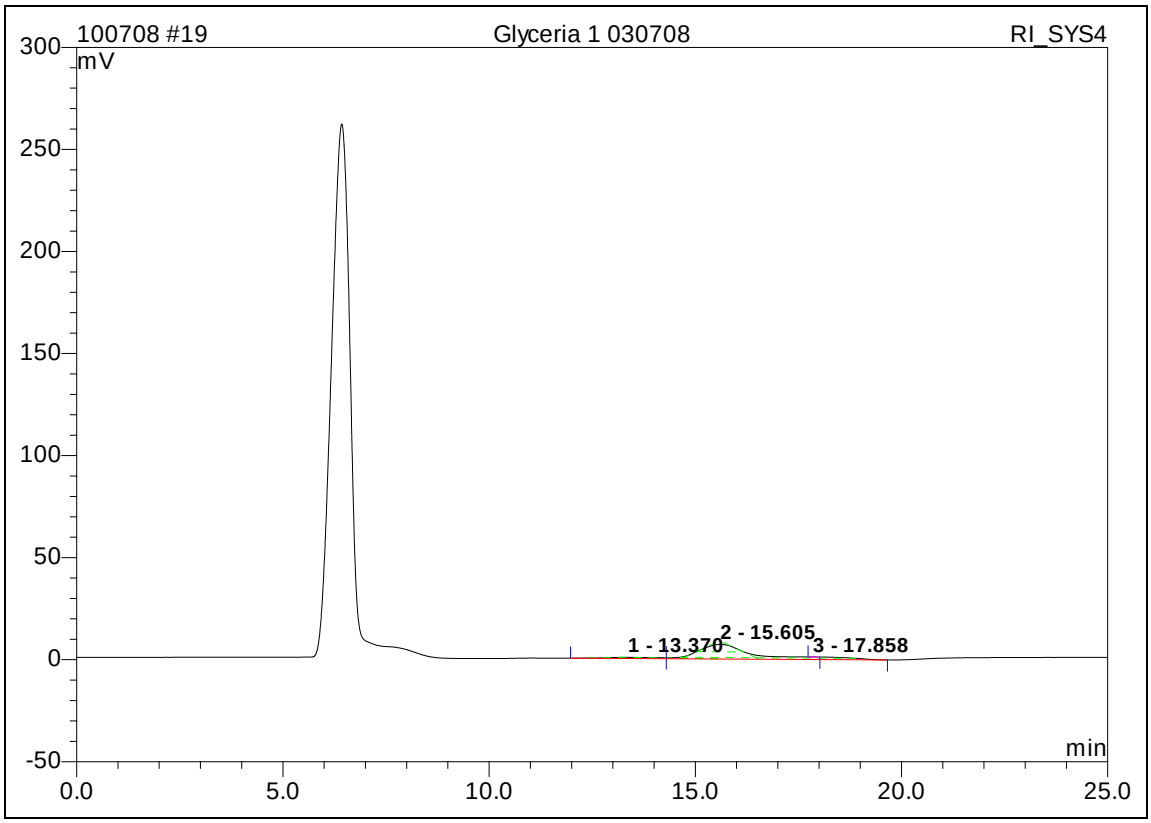

Fig. 33 Peak result of Reed mannagrass representative sample from the fifth week analyzed on HPLC.

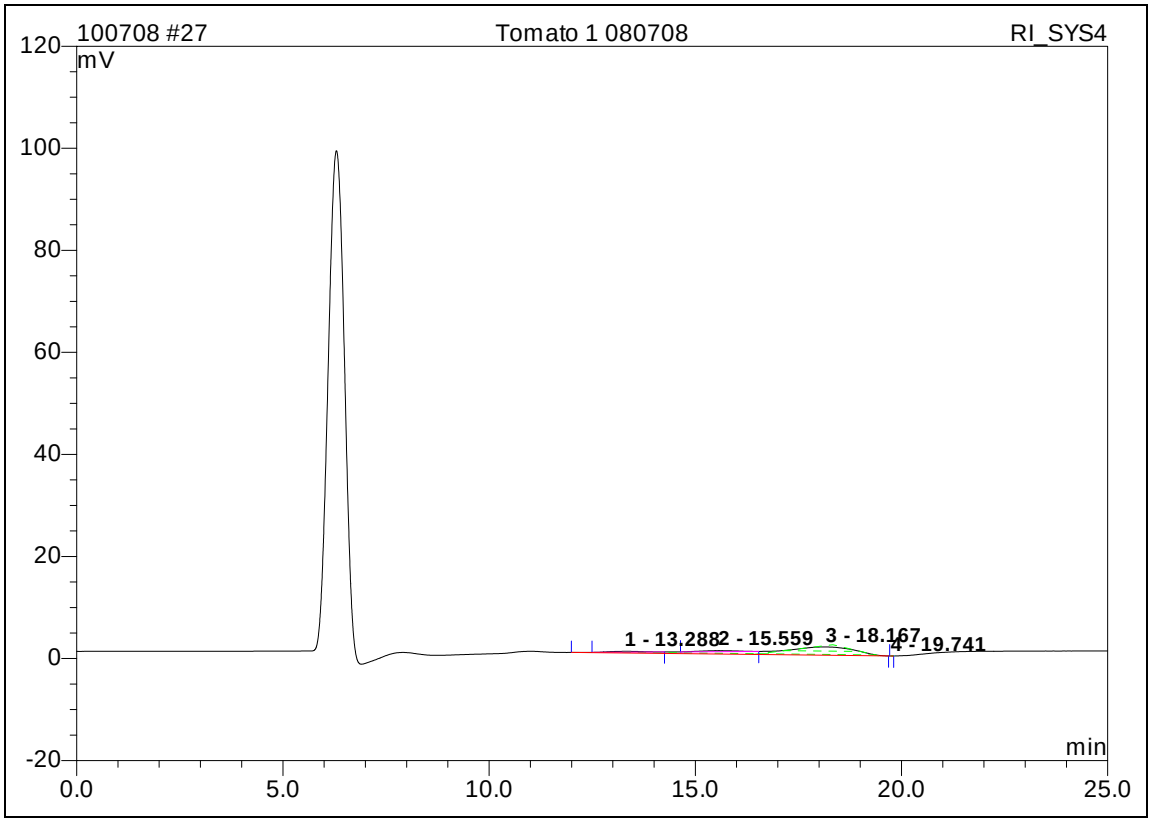

Fig. 34 Peak result of Reed mannagrass representative sample from the sixth week analyzed on HPLC.

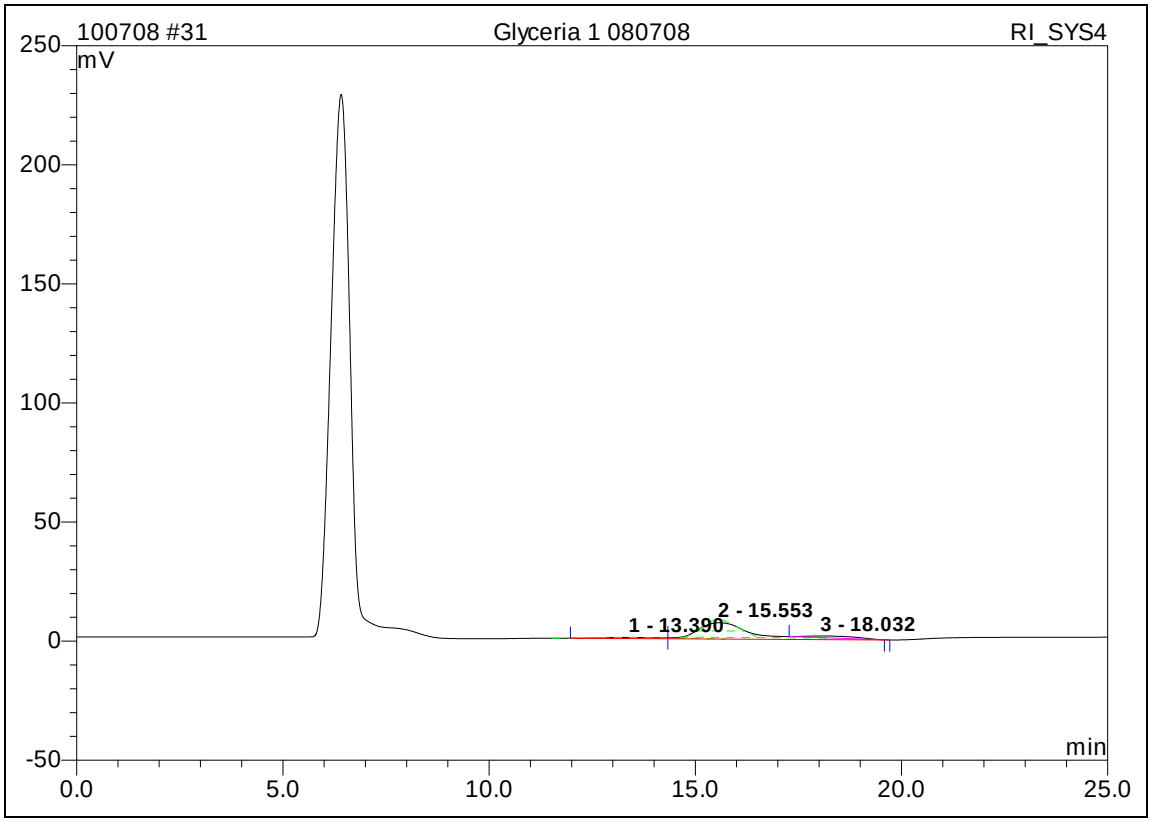

Fig. 35 Peak result of Reed mannagrass representative sample from the sixth week analyzed on HPLC.

Consequences of anaerobic root growth

After 3 weeks from the start of the experiment observations were made to the plants that were suffering from anoxia and growth stop. It seems more reasonable that the symptoms as presented in Figure 36 are associated with severe auxin or cytokinin overdosing as a result from the anaerobic root growth and the collection of high concentrations of sugars inside the plant due to the remove of flowers.

Figure 36. Multiple pictures of subsequent observations when keeping the tomato plants anaerobic in the root environment and pinching all shoots and trusses. From left to right we can see: 1.Dark leaves and downward curving leaves. 2. Bursting of epidermis. 3. Starting adventitious roots on the lower stem. 4. Shoots on the leaves. 5. 10-20 shoots at previously pinched shoots on the stem. 6. Adventitious roots bursting through the epidermis higher on the stem, eventually up to the top.

Effect of bacterial metabolism during sample incubation

The objective of this investigation was to determine if there is organic acid or sugars reduction caused by bacterial metabolism in samples after restoring them from -18 ^{o}C. Samples were collected from several plants of reed mannagrass rhizosphere and stored at -18 ^{o}C . Several days after , the samples were allowed for 3 hours to restore their liquid form at room temperature. To compare possible reduction of exudates in our samples after the restoration additional nutrient solution was prepared in high concentration made with saccharose (5%) and citric acid (5%) in 100ml water

to mix with the samples we took form reed mannagrass rhizosphere . Finally thirteen samples were prepared of 1 ml each , where twelve of them contained ½ prepared nutrient solution and ½ sample from reed mannagrass rhizosphere with bacteria. One standard sample was used with ½ prepared nutrient solution and ½ water . Each sample was tested on different time to determine the reduction of sugars or organic acids over time.

Results

At the first sample that was analyzed 45 minutes after the standard solution a reduction of 5.000 mg/L of saccharose was detected. Sample 2 after 90 minutes had also some sugar reduction showing 1.100 mg/L less than the standard. Sample 3 and sample 6 had the most saccharose reduction. In average all samples had lost 5.000 mg/L. Citric acid had the same concentration level in every sample until the end of the experiment.

Table 4.Effects of reed mannagrass samples with bacteria to the concentrations of organic acids and sugars standard solution

Sample	T analysis(min)	Saccharose mg/L	Citric acid mg/L
Standard	0	50.000	50.000
1	45	45.400	50.000
2	90	48.900	50.000
3	135	41.100	50.000
4	180	45.100	50.000
5	225	47.500	50.000
6	270	38.700	50.000
7	315	45.000	50.000
8	360	45.100	50.000
9	405	45.000	50.000
10	450	45.000	50.000
11	495	44.800	50.000
12	540	45.100	50.000

As shown on Table 4 there is reduction of saccharose in the amount of 5.000 mg/L in average which is very high for 1 ml samples. As for the citric acid it is mentioned in the literature [1] that specifically bacteria that grow in tomato and cucumber rhizosphere substrate grow better than other bacteria randomly selected and when the citrate is the main carbon and that can explain why there was no reduction noticed in the samples.

In the process of analyzing samples with the HPLC method there is a period of time before the actual analysis where bacterial presence can affect the results by metabolizing the substrate. The samples taken from the reed mannagrass rhizosphere contain bacteria that survive after -18oC and they become active again while the samples stay in room temperature. The bacteria as it seems consumed up to 5.000mg/L in 3 hours and 45 minutes (5mg/ml which is the vials of HPLC analysis).

Citric acid identified as the peak at 10.200 min. The double peak that follows is saccharose which is recognized as glycose and fructose together as for it is a disaccharite at 11.300min and 12.000min. We can see the reduction of Saccharose (fructose with glucose) while citric acid remains the same amount.

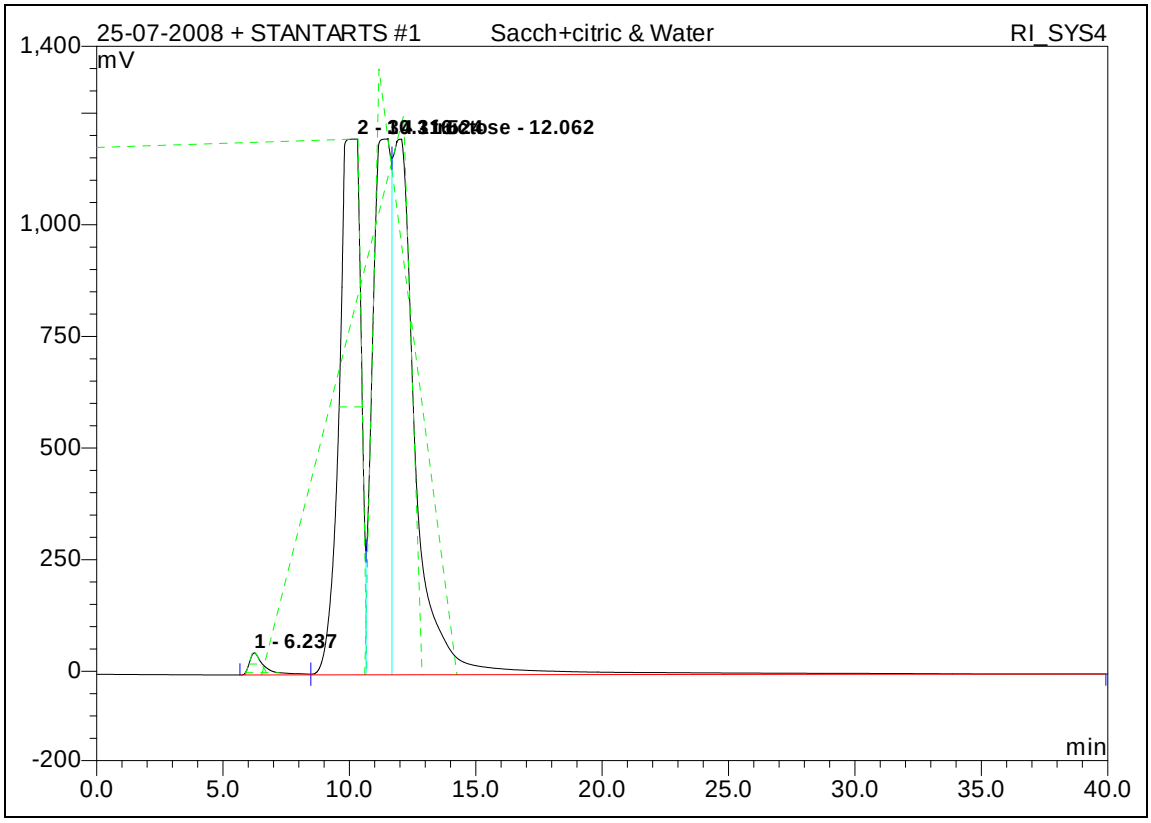

Fig. 1. Saccharose mixed with citric acid and water as a stantart analysed at 0 min

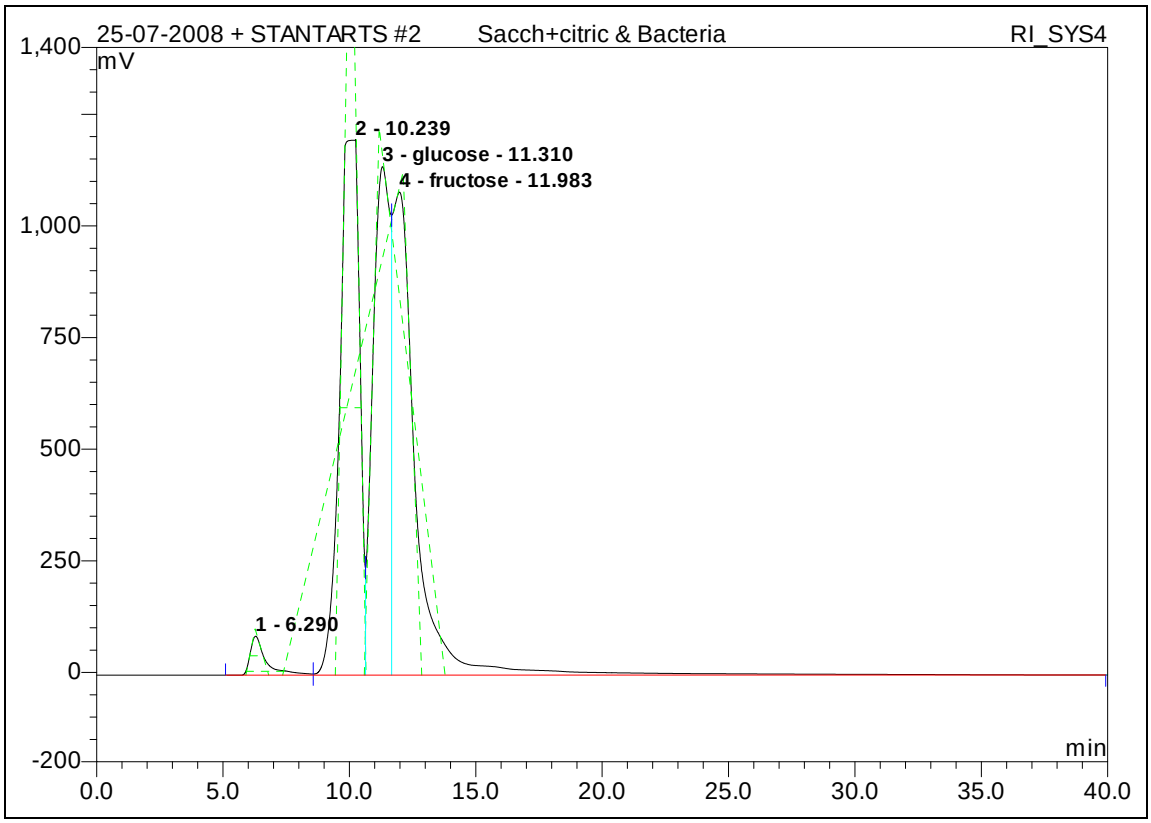

Fig. 2. Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 45 min

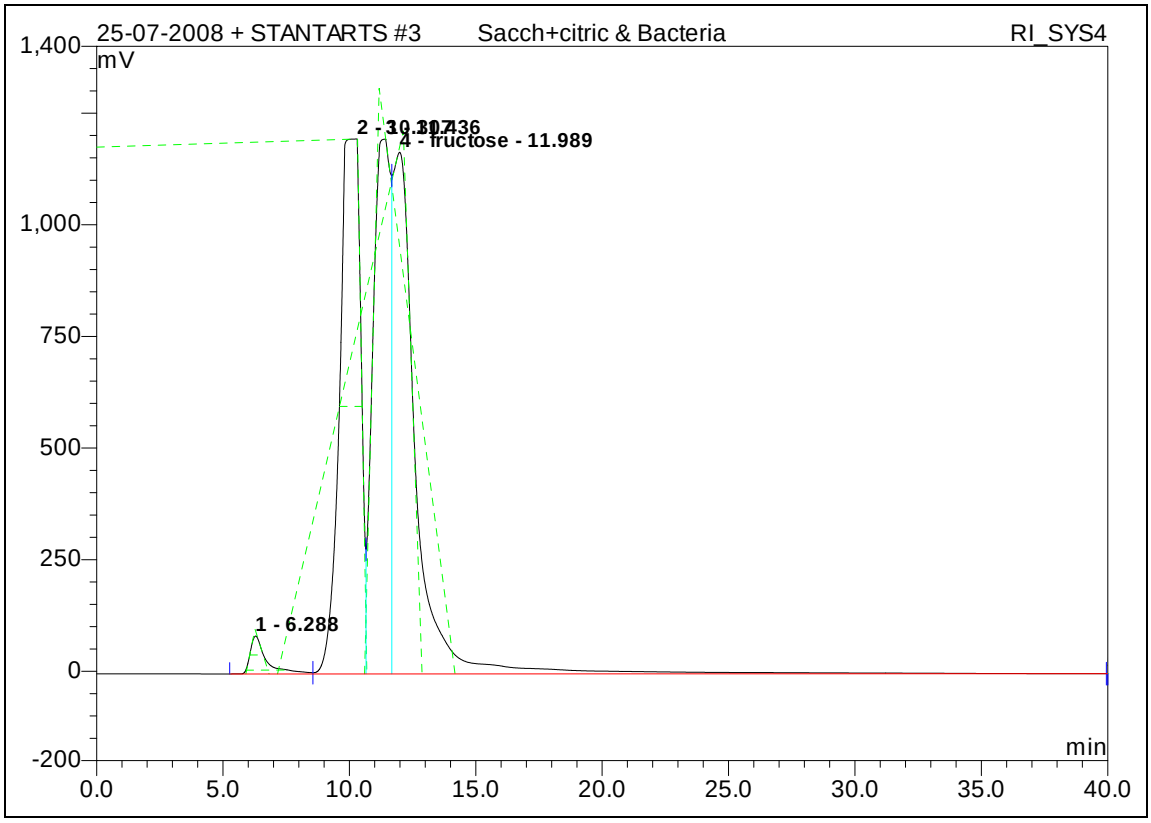

Fig. 3. Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 90 min.

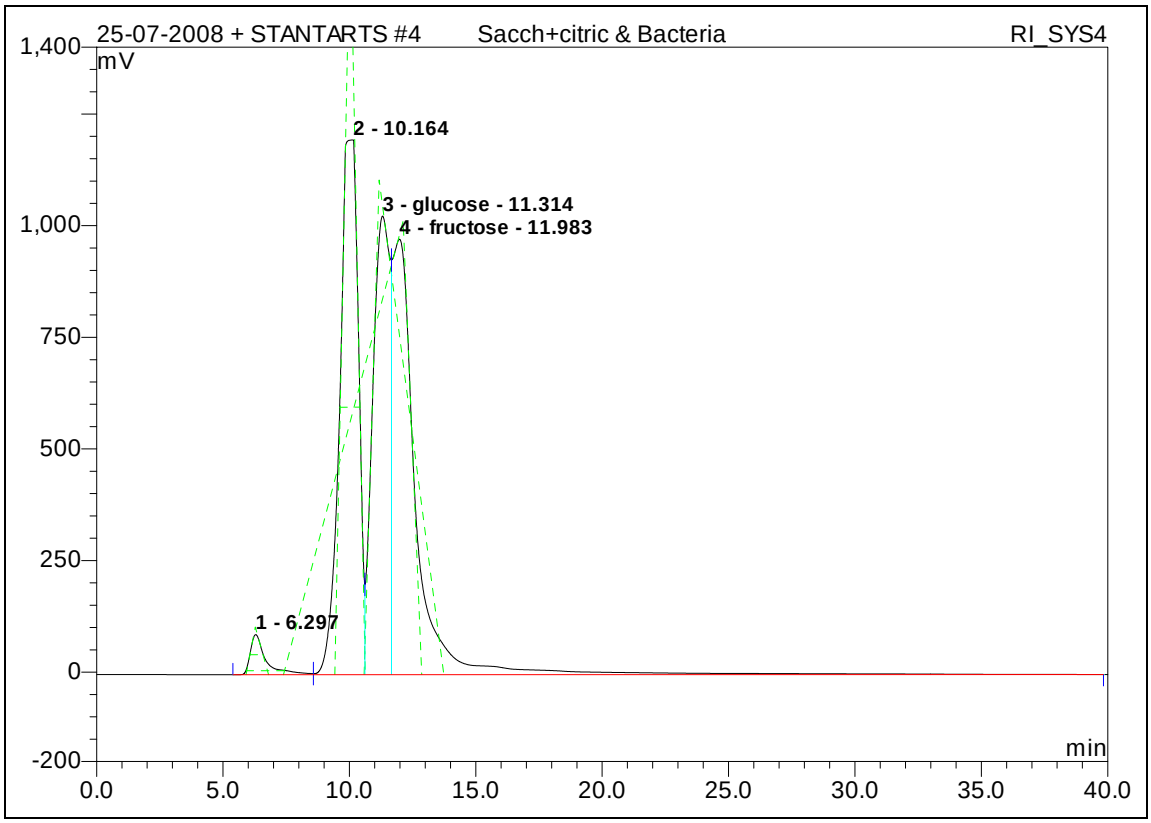

Fig. 4. Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 135 min.

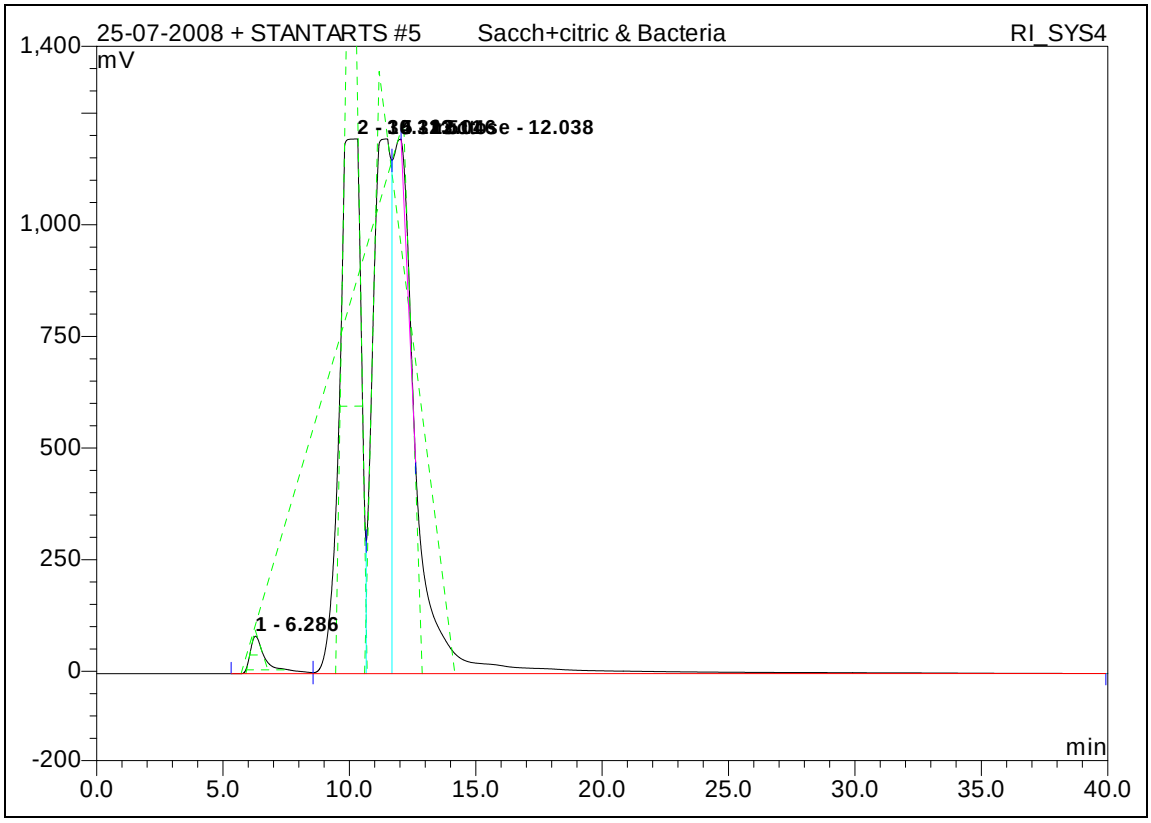

Fig. 5. Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 180 min.

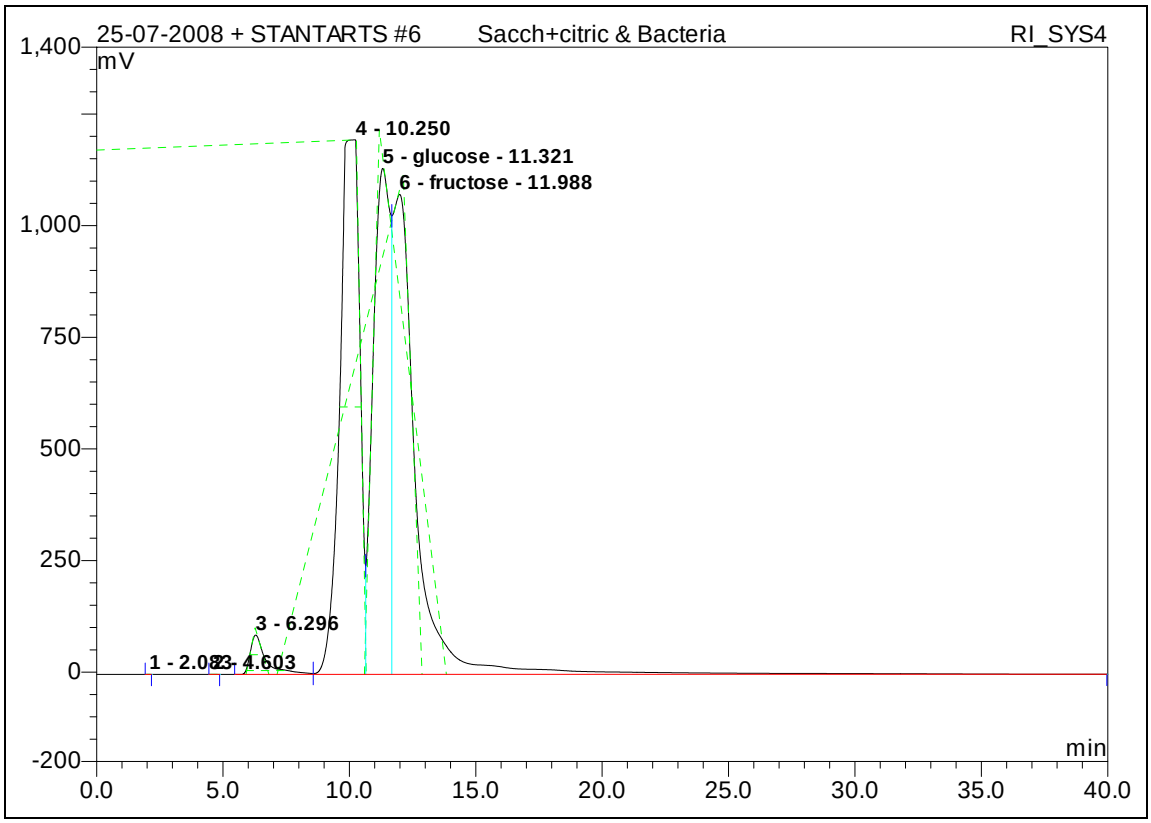

Fig. 6. Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 225 min.

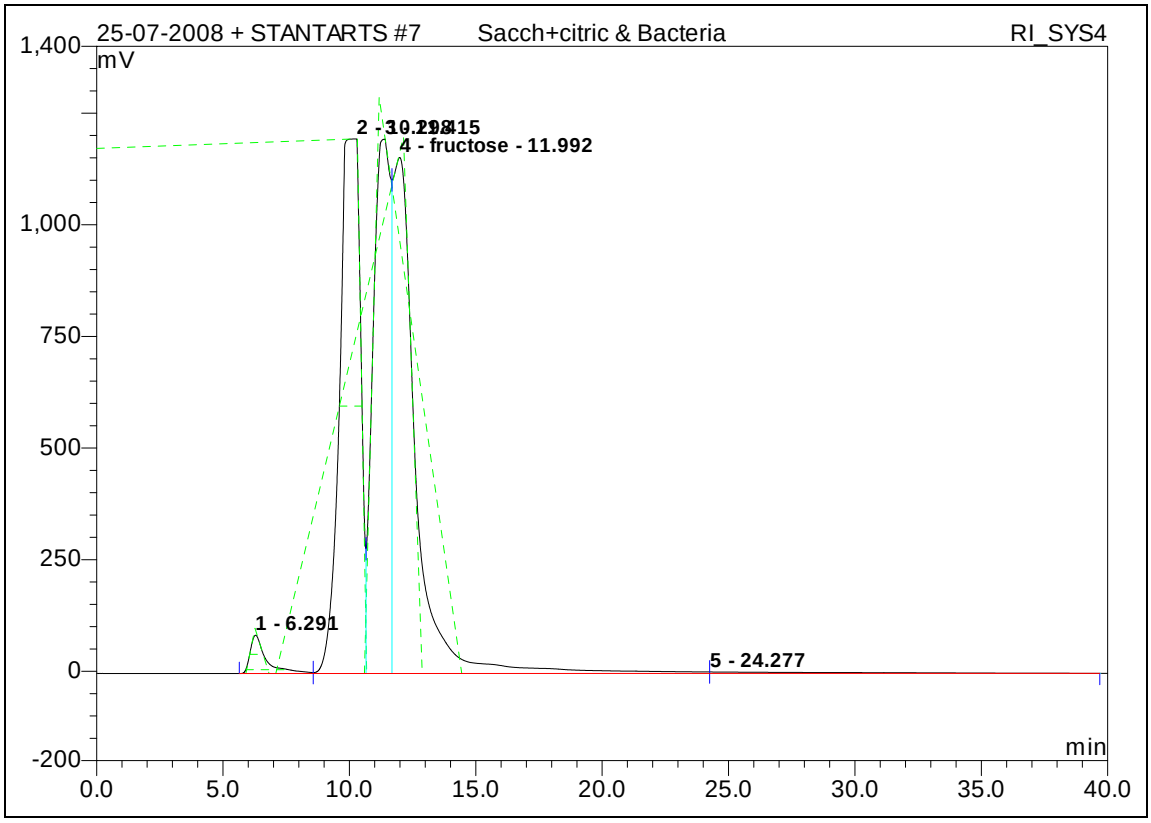

Fig. 7 . Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 270 min.

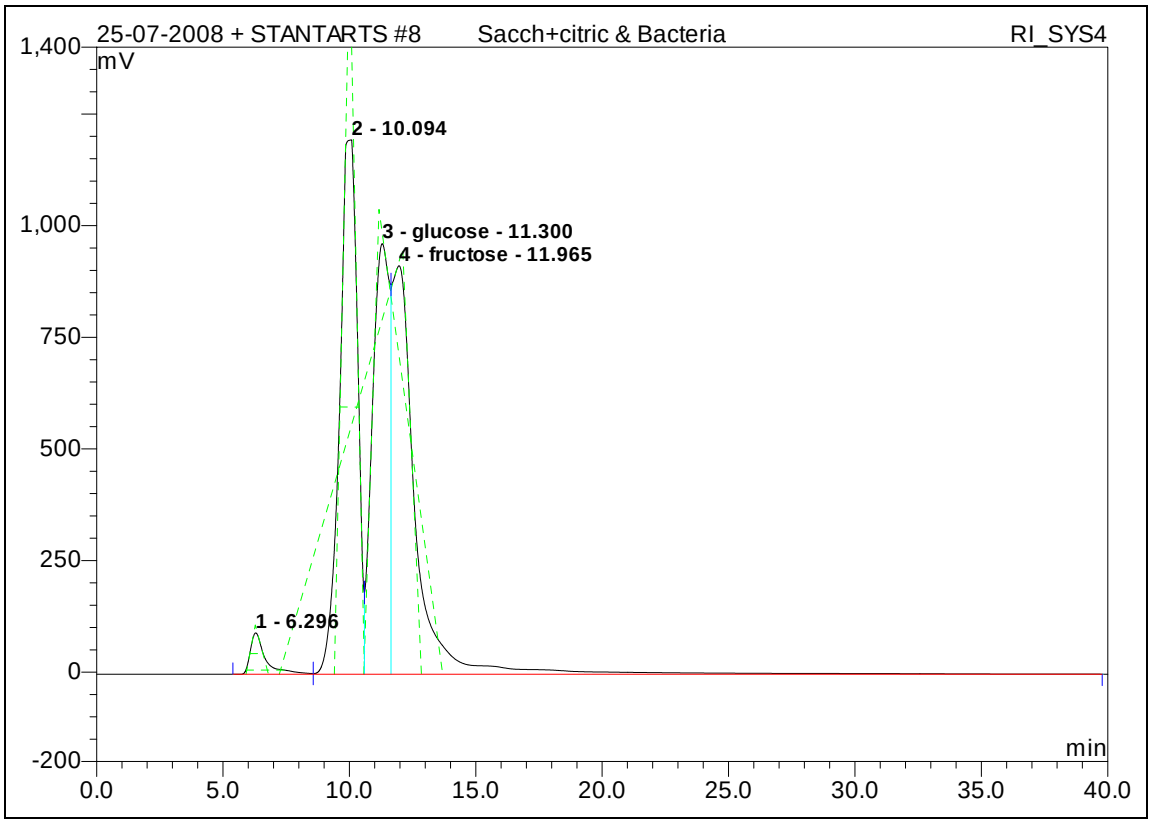

Fig. 8 . Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 270 min.

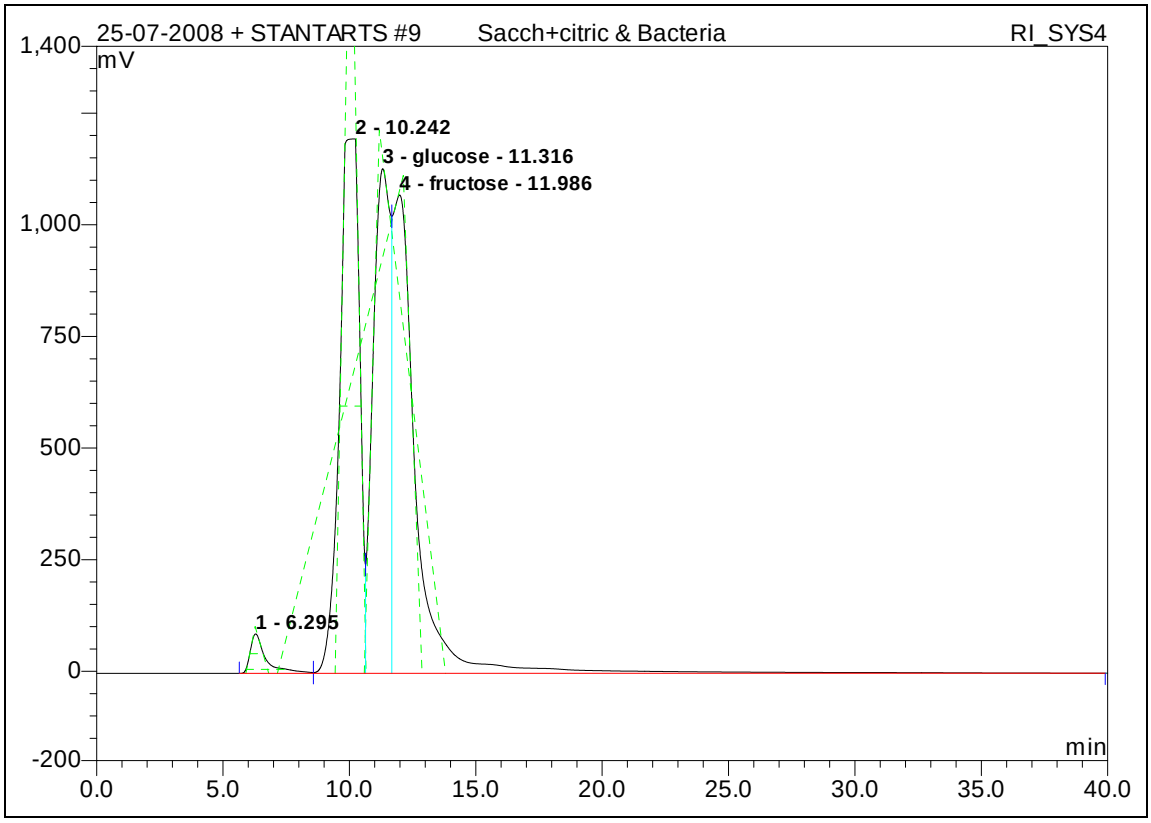

Fig. 9 . Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 315 min.

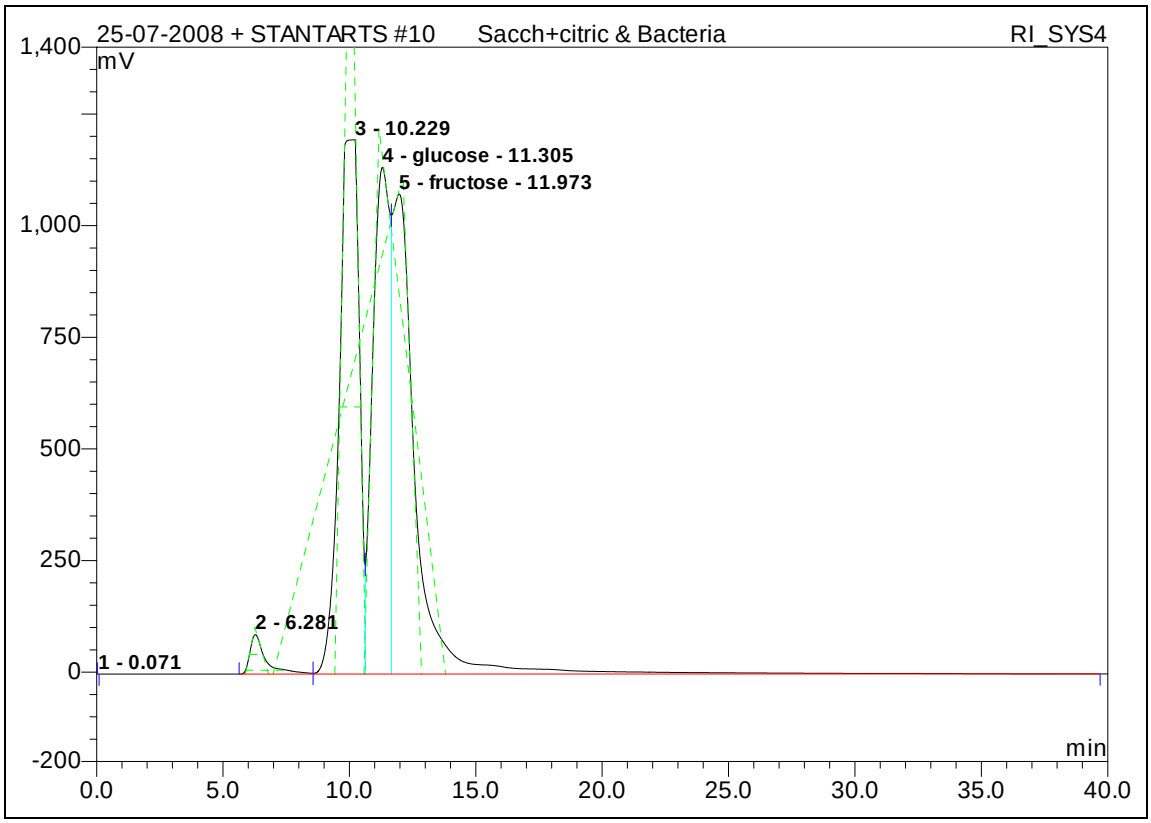

Fig. 10 . Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 360 min.

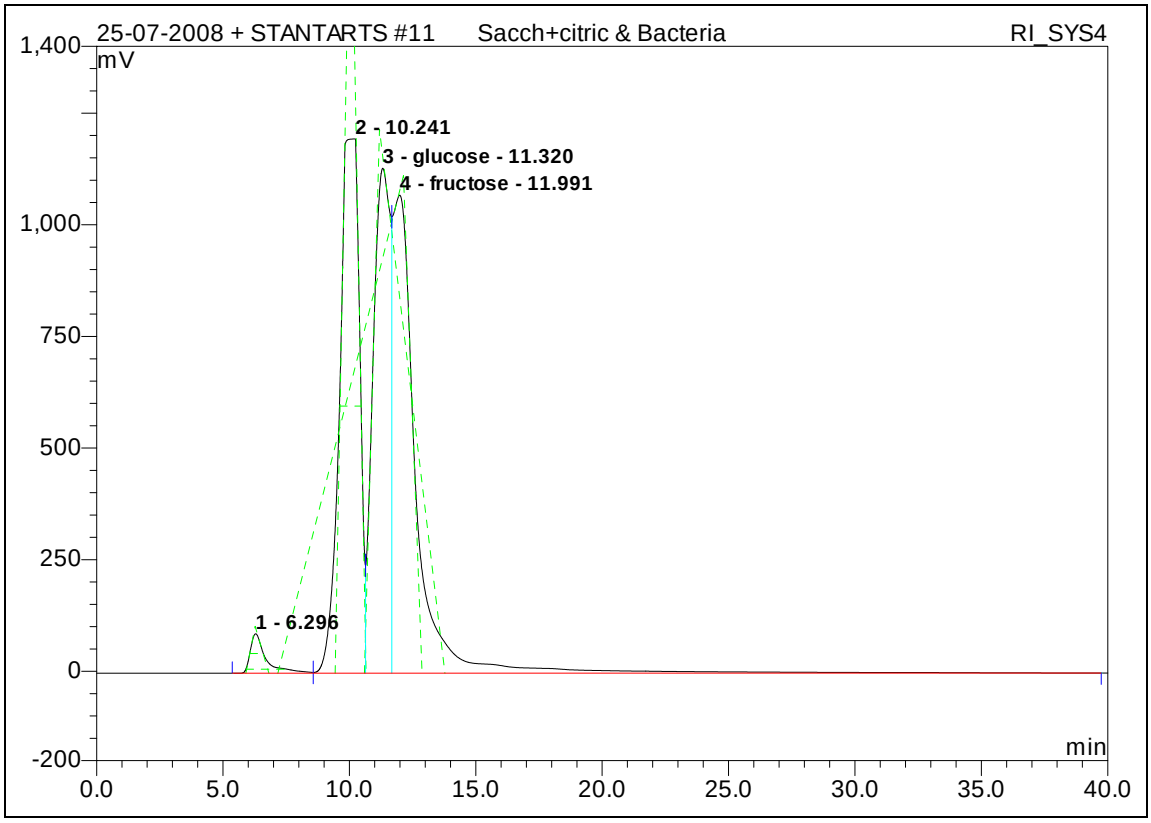

Fig. 11 . Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 405 min.

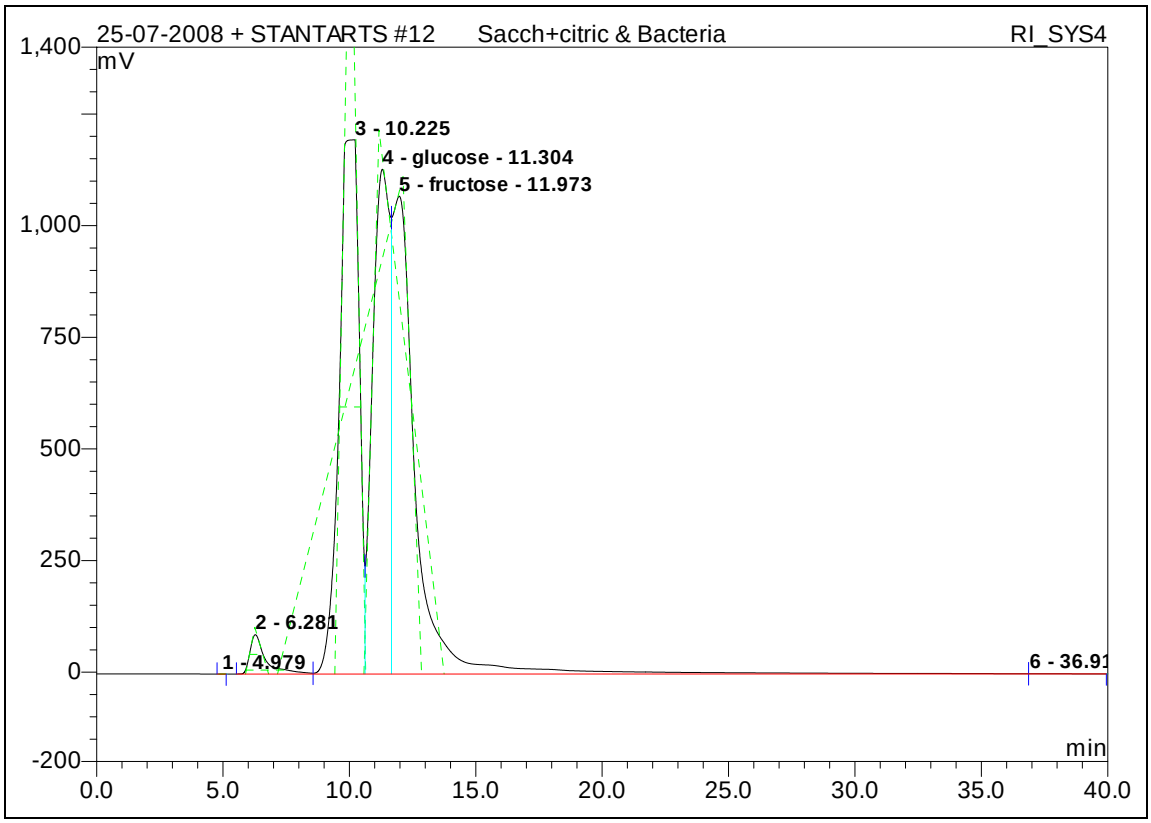

Fig. 12 . Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 450 min.

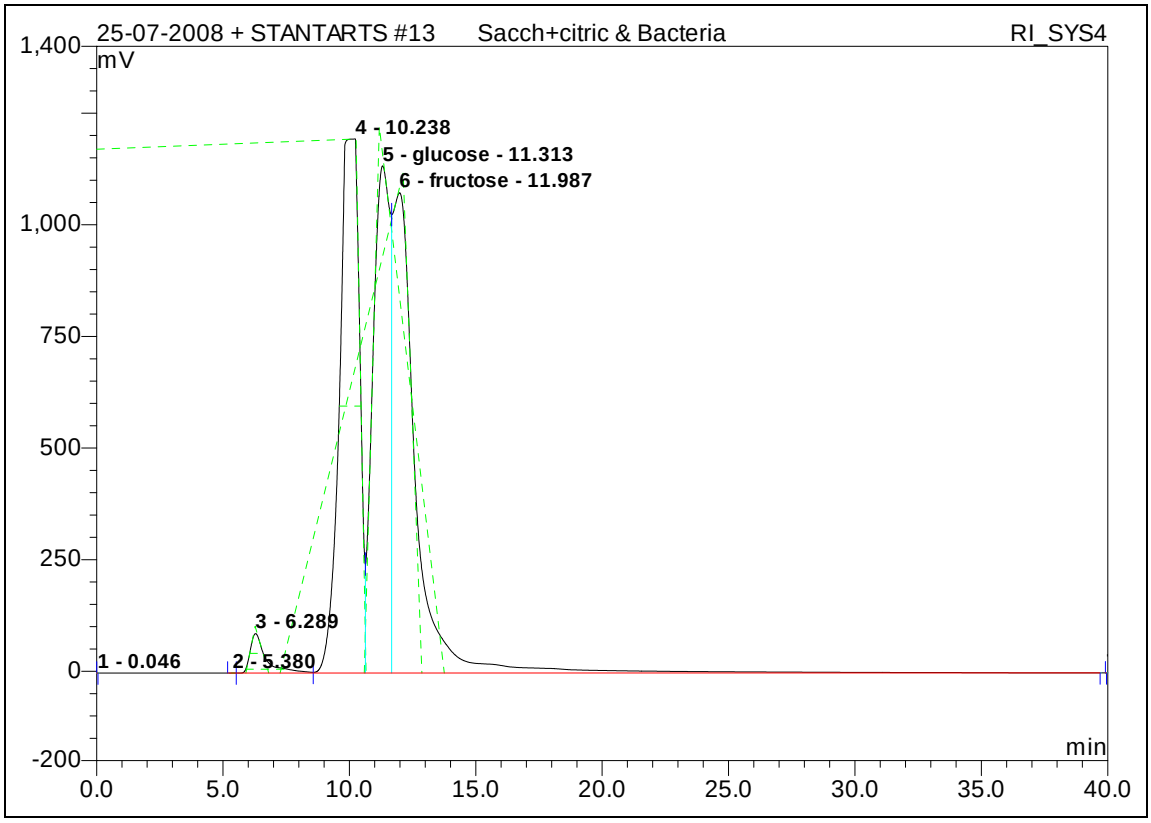

Fig. 13 . Saccharose mixed with citric acid and sample taken from Glyceria plant analysed at 495 min.