# Bioponics for lettuce production in plant factory with artificial lighting

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## 1. Abstract

Plant Factory with Artificial Lighting (PFAL) gains worldwide attention and the most popular culturing system is hydroponics which pure soluble inorganics fertilizers were used. The manufacturing process of these fertilizers are not environmental friendly. A new trend termed 'Bioponics' using organic fertilizer brought to our attention. This study aims at using two organic fertilizers (BIO N and BIO NK, Swiss Hydroponics Lab.) in a DFT system in PFAL producing Frill-ice lettuce, Boston lettuce and Frilly lettuce. Using Yamazaki formula (inorganic fertilizer) as the control group. The environmental settings of the PFAL are as follows: PPFD kept at 200 μmol $m^{-2}s^{-1}$, day/night temperature at 25/18℃ and $CO_2$ concentration at 1200 ppm. Results showed that the control group has the highest fresh mass in Frill-ice lettuce. Bioponics treatments greatly reduced the nitrate concentration of all lettuce and increased the fresh mass of Frilly lettuce. BIO N is not suitable for Boston lettuce. Further studies will be conducted using BIO NK and the Frill-ice lettuce. Using organic fertilizer in PFAL looks promising, however, the products of Bioponics can be considered organic products or not is still in controversy.

**Keywords:** Bioponics, Hydroponics, Deep Flow Technique, Organic

## 2. INTRODUCTION

Soilless culture is a widely and frequently used technique to grow plant without soil. It can use organic or inorganic substitute materials such as bark or rockwool or non-solid materials. Hydroponics is subset of the soilless culture using circulated nutrient solution. It provides a considerable degree of control of the elemental environment surrounding the root. Hydroponic cultivation systems have been developed to control soil-borne pathogens (Uyeda, et al., 2011) and especially powerful in element-deficit research. The ability to use organic fertilizer in hydroponics has been studied as a method to grow crops in space habitats (Garland, et al., 1997). The results showed direct use of organic fertilizer proved to be deleterious to plant growth.

Due to environmental protection and food safety reasons, organic agriculture bring lots of public attention. Many people considered organic agriculture can also provide more nutritious food with better taste. It is controversial. The National Organic Standards

Board(NOSB) is a Federal Advisory Board in the USA made up of 15 dedicated public volunteers from across the organic community. In 2016, NOSB announced a definition for the term 'bioponics' as below:

*Bioponics is a contained and controlled growing system in which plants in growing media derive nutrients from plant-based, animal-based and mineral natural substances which are released by the biological activity of microorganisms.*

Aquaponics and organic hydroponics fit the definition of bioponics. There are three major roles in aquaponics: fish/prawn, plant and beneficial bacteria. The fish/prawn wastes and uneaten feeds contained ammonia, which dissolved in water becomes ammonium ion, which can be nitrified by bacterial to become nitrate ion and nitrite ion. Both ammonium and nitrate ions are nutrient to the plants.

Traditional hydroponics use inorganic fertilizer in the circulated nutrient solution, organic hydroponics replace inorganic fertilizer with organic fertilizer which maybe combination of extracts from agriculture waste and/or plants/animals.

Shinohara, et al. (2011) compare the production of hydroponically grown tomato using organic and inorganic fertilizers. Results appeared that roots grown with inorganic fertilizer had no root hairs and large numbers of root hairs developed on the roots of tomato grown with fish-based soluble fertilizer. The hairs were also covered with biofilms Organic hydroponics can also induced systemic resistance (ISR) in lettuce against air-borne Botrytis cinerea, which causes gray mold (Chinta, et al., 2015). It can be stated that organic fertilizer can enhance/induce the growth of root hair thus providing better absorption of nutrients and less air-borne bacterial induced diseases, leading to better growth and yields.

The production of inorganic fertilizer from mineral to powder required lots of energy and creates lots of pollutants. The shipping and transporting of inorganic fertilizer from manufacturing sites to the sites of plant growth also required lots of energy thus it is non-environmental friendly compare with locally produced organic fertilizer. Most of the PFAL (Plant Factory with Artificial Lighting) uses multi-layer shelf equipped with hydroponic system such as NFT (Nutrient Film Technique), DFT (Deep Flow Technique) or E&F (Ebb and Flood). In such system, inorganic fertilizer are used. Recently, organic fertilizer was tested which also brought lots of attention. The purposes of this study was to test on three varieties of lettuce using two types of commercially available organic fertilizer in PFAL. To be used in confined environment such as PFAL, any material with bad smell is not allowed, thus leading to organic fertilizers extract from plant-waste a better choice.

## 3. MATERIALS AND METHODS

### Culturing condition

Three varieties of lettuce (*Lactuca sativa* L.), namely Frill-ice, Boston, and Frilly

were grown in the PFAL of National Taiwan University. All seeds are locally available. As shown in Table 1 is the treatment code of 3 stages: pre-treatment stage, seedling stage and mature stage. Treatments of nutrient solution are: Purpose for the pre-treatment stage is to culture the water, to allow the growth of nitrifying bacterial in order to reduce the concentration of $NH_4^+$ and increase the concentration of $NO_3^-$. It required 21 days before sowing (DBS).

Inorganic fertilizer Yamazaki formula was used as the check and two organic fertilizers, BIO N and BIO NK (trade name of Swiss Hydroponics Lab) were tested in this study. Three types of lettuce were grown hydroponically from sowing to harvest for 42 days (Frill-ice lettuce) and 35 days (Boston and Frilly lettuce), fresh weight and minerals within the plants were analyzed.

Table 1 Treatments and codes in stages of lettuce production with organic fertilizer

| Treatments | Pre-treatment stage<br>DBS 21 | Seedling stage<br>DAS 0-14 | Mature stage<br>DAS 15-35/42 |
|---|---|---|---|
| CK | - | N1_E1.2_p6.0_L200_<br>d918_H24_A25 | N1_E1.2_p6.0_L250_H16_<br>d27_A25/18 |
| BIO N | N2_E0.4_W23_<br>DO8 | N2_E0.4_p6.0_L200_<br>d918_H24_A25 | N2_E0.4_p6.0_L250_H16_<br>d27_A25/18 |
| BIO NK | N3_E0.4_W23_<br>DO8 | N3_E0.4_p6.0_L200_<br>d918_H24_A25 | N3_E0.4_p6.0_L250_H16_<br>d27_A25/18 |

Note: DAS: Days After Sowing
DBS: Days Before Sowing
Treatment code:
Nx: N, Nutrient solutions
N1: Yamazaki Nutrient solution recipe
N2: BIO N (Swiss Hydroponics Lab)
N3: BIO NK (Swiss Hydroponics Lab)
$E_x$: E, Electrical conductivity (EC) of nutrient solution,
x, Value of EC, Unit: $mS \cdot cm^{-1}$.
px: p, pH value
$L_x$: L, LED tube (cool white, color temperature 6500 K)
x : Value of light intensity, Unit : $\mu mo\ l \cdot m^{-2} \cdot s^{-1}$.
dx: d, Cropping density,
x, Value of cropping density, Unit : $plts \cdot m^{-2}$
$H_x$: H, Duration of light period,
x : Hours of light period per day, Unit : $\cdot hours\ day^{-1}$
A dT/nT: A, average day/night temperature, Unit : $^oC$.
W: W, Water temperature, Unit : $^oC$
DOx: DO, Dissolved oxygen, Unit : ppm.

**Methods of measurement**

Fresh weight of the plant

Remove roots and sponge, measured using electronic scale.

Nitrate and ammonium contents in nutrient solution

Reflectometer (Rqflex 10, Merck) with nitrate and ammonium test papers

Mineral contents within the plants

Take complete leaves, take 1g, frozen at -20 ° C for 48 hours grinding extraction dilution to the appropriate concentration (He et al., 1998). The samples were analyzed for their anion and cation contents using an ion analyzer (IA-300, DKK-TOA Corporation, Japan, Cationic column: PCI-205l, Anion column:PCI-322).

Statistical analysis

The results were analyzed using Duncan's multivariate analysis using statistical software SAS 9.1. Differences were considered significant when $p < 0.05$.

## 4. RESULTS and DISCUSSION

Saijai et al.(2016) used composted bark waste as the organic fertilizer in the water, pretreated for 21 days and found two beneficial bacterials: ammonia-oxidizing bacteria (Nitrosomonas) and nitrite-oxidizing bacteria (Nitrobacter) were involved in the conversion from $NH_4$ to $NO_2$, finally to $NO_3$. Shinohara et al. (2011) found that in some aquaponics system failed to grow lettuce seedling, maybe due to too much ammonium in the water. That is lack of pre-treatment stage. Same conclusion was revealed in an early study by Atkin and Nichols (2004), they concluded that in an organic hydroponics, with only ammonium-nitrogen without nitrate-nitrogen will significantly suppressed lettuce growth.

As mentioned above, organic fertilizer in nutrient solution required pre-treatment to allow ammonia- and nitrite-oxidizing bacterial to grow. As shown in Fig. 1 and 2, the ammonium concentration increases in the beginning and start to decrease at day 14, while the nitrate concentration starts to increase. Nitrate concentration of BIO NK on day 21 is much higher than the nitrate concentration of BIO N on day 25.

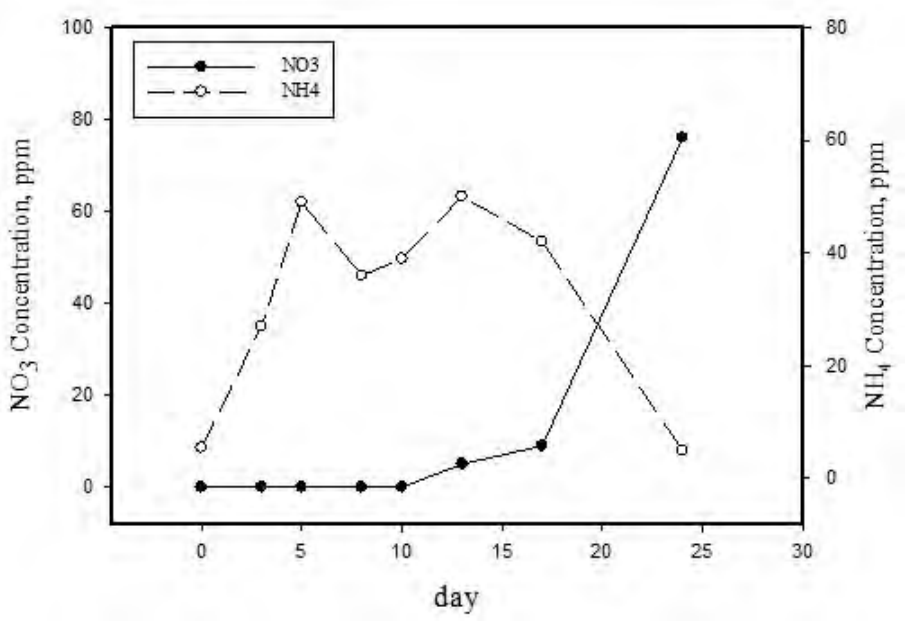



Fig. 1 Changes of nitrates and ammonium concentration in organic fertilizer (BIO N) during the pre-treatment stage

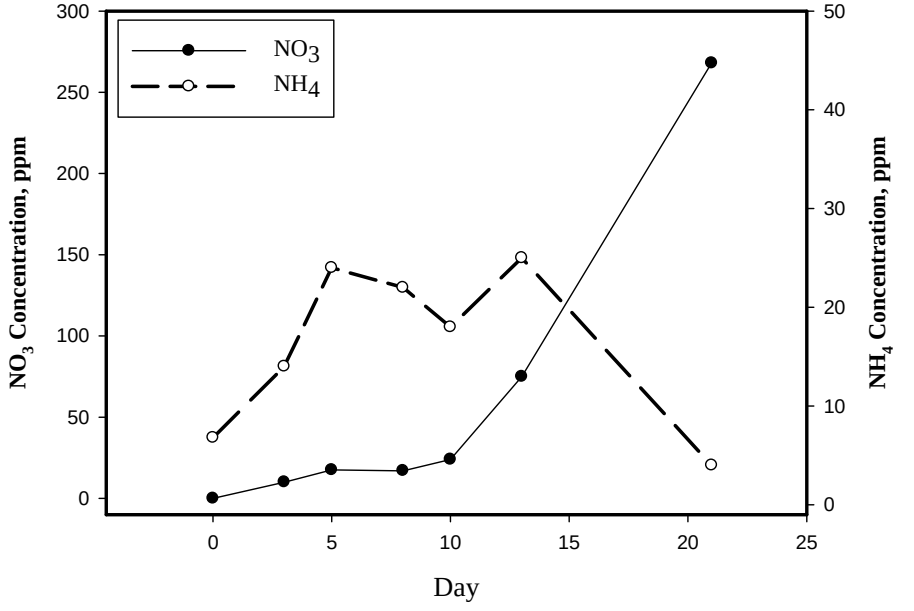



Fig. 2 Changes of nitrates and ammonium concentration in organic fertilizer (BIO NK) during the pre-treatment stage

Fig. 3 shows three types of lettuce harvested under three types of nutrient solutions. Organic fertilizers seems to suppress the growth of Frill-ice lettuce. The leaf color seems to

be lighter on Boston and Frilly lettuce grown using organic fertilizer. Frilly lettuce prefer BIO NK with highest fresh weight and Frill-ice prefer Yamazaki formula. Lettuce grown using inorganic fertilizer leads to higher nitrate content in the leaf and low nitrate content can be expected when grown using organic fertilizers as shown in Table 2. Similar results were also found by Shinohara et al. (2011). Reason might due to interaction between plant and microorganism. In most cases, plants grows slowly with organic fertilizer compare with plants grow with inorganic fertilizers, however, the human health benefit of greens with lower cellular nitrate-nitrogen may outweigh minor differences in yield as stated. This study with the add-in pre-treatment stage, as shown in Table 2 and Fig. 3, the fresh weight can be higher and merit of low nitrate concentration can be remain. Hanafy Ahmed et al. (2000) found that bio-fertilizers can not only reduce the nitrate content but also increase total sugars, total free amino acid and total soluble phenols of lettuce. It is suggested that plants may use the accumulated nitrate as an osmoticum and enables it to use more carbohydrates for plant structural growth, thus increasing the dry matter.

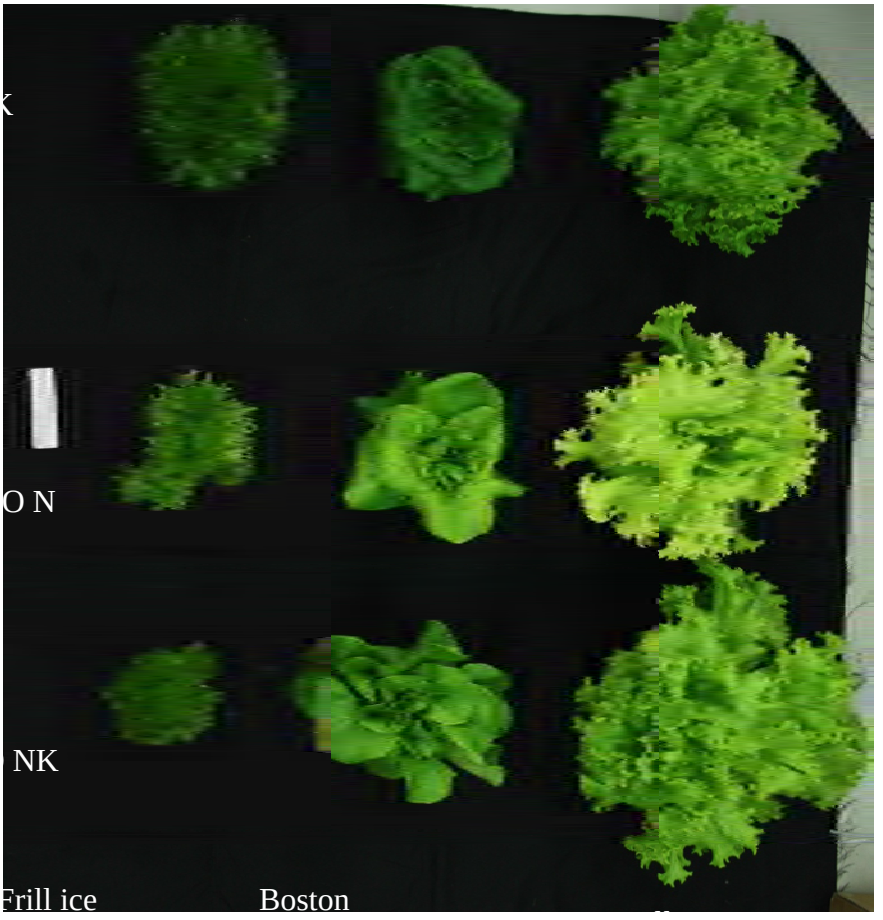



Fig. 3 Effects of different nutrient solutions on the growth of three types of lettuce (Frill ice lettuce, DAS 42. Boston and Frilly Lettuce, DAS 35. Bar = 10 cm).

Table 2 Effects of different nutrient solutions on fresh weight and nitrate content of three types of lettuce

| | Fresh weight (g) | | | Nitrate content (ppm) | | |
|---|---|---|---|---|---|---|
| | Frill ice | Boston | Frilly | Frill ice | Boston | Frilly |
| CK | 82.3 a | 88.8 a | 75.0 c | 6113.5 a | 3251.4 a | 5449.0 a |
| BIO N | 23.1 b | 64.1 b | 108.3 b | 888.4 c | 1333.6 b | 1159.0 c |
| BIO NK | 25.0 b | 92.1 a | 176.1 a | 1527.4 b | 3908.7 a | 2016.4 b |

Means followed by the different letters in each column are significantly different at 5% level by Duncan's Multiple Range Test. n=10.

As shown in Table 3, different nutrient solution has no impact on ammonium contents in the leaf of lettuce. Organic fertilizers lead to higher concentration of Na, Ca and Mg in leaf of lettuce. BIO NK leads to higher potassium in the leaf compare with BIO N. Lettuce grown in BIO NK can have similar concentration of potassium ions in the leaf compare with lettuce grown using Yamazaki formula. As shown in Table 2, the fresh weight of lettuce grown using BIO NK is much higher than grown using BIO N. Reasons might be not only BIO NK provides more nitrate but also higher potassium and less sodium concentrations. Organic fertilizers can be further improved by reducing the concentration of sodium.

Table 3 Effects of 3 nutrient solutions on various ions in leaf of 3 types of lettuce

| Treatments | Lettuce cultivars | $NH_4$ (ppm) | Na (mg/100g) | K (mg/100g) | Ca (ppm) | Mg (ppm) |
|---|---|---|---|---|---|---|
| CK | Frill ice | 7.4 a | 2.9 d | 384.5 b | 253.6 c | 98.8 c |
| | Boston | 7.6 a | 3.0 d | 412.5 ab | 234.8 c | 73.5 c |
| | Frilly | 15.0 a | 3.4 d | 418.0 ab | 328.0 bc | 87.2 c |
| BIO N | Frill ice | 9.1 a | 18.6 b | 170.8 d | 840.0 a | 291.3 a |
| | Boston | 10.8 a | 26.5 a | 262.0 c | 946.7 a | 296.3 a |
| | Frilly | 16.8 a | 19.3 b | 138.8 d | 567.5 b | 216.7 ab |
| BIO NK | Frill ice | 10.0 a | 8.8 c | 298.8 d | 192.7 c | 71.7 c |
| | Boston | 10.5 a | 19.7 b | 484.0 a | 550.3 b | 185.6 b |
| | Frilly | 10.3 a | 16.6 b | 403.5 ab | 546.7 b | 147.7 bc |

Means followed by the different letters in each column are significantly different at 5% level by Duncan's Multiple Range Test. n=5.

## 5. CONCLUSIONS

Using organic liquid fertilizer in PFAL is quite promising for semi-head and leafy green production. Enhance the growth of root hair, reduce the nitrate concentration were proof by previous researchers and this study. This study further proof that with proper pre-treatment, without scarifying the fresh weight of the produces is possible. The environment in a PFAL is relatively clean. The plant-microorganism relationship can be proper managed to ensure efficient use of organic fertilizers. Most of the organic fertilizers can be locally produced and extracted from agricultural wastes, thus making the organic hydroponics more environmental friendly compare with traditional hydroponics using chemical fertilizers. In two types of organic fertilizer tested, BIO NK is preferred compare with BIO N due to rapid conversion from ammonium to nitrate, rapid growth of lettuce as well as proper concentration of minerals in lettuce. Organic fertilizers can be further improved by reducing the concentration of sodium. The need for pre-treatment stage seems to be the constraint of using organic liquid fertilizer if production time is of great concern. More study on add-in beneficial bacterial in organic hydroponic to reduce the duration of the pre-treatment time will be conducted.

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