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Research on some functions of Azolla in CELSS system

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Abstract

This article detailed the possibility of using Azolla in CELSS system, the characters of Azolla; the experiments on using Azolla as O_2-releasing plant to provide O_2 for human in airtight chamber; using Azolla as an important biological part for urine solution purification was also introduced.

Keywords: O_2 provision; Photosynthetic O_2 release; CELSS system; Shade tolerance; PPO; Urine purification; Photocatalytic oxidation; TiO_2 plating sheet

Azolla is a small aquatic fern which floats on the water surface. It contains within its leaf cavities a symbiotic cyanobacterium–*Anabaena azolae*. The N-fixing capacity of *Anabaena azolae* enables Azolla to thrive on nitrogen-free waters.

Azolla is historically cultured in the rice-farming system in China and Southeast Asian countries. The fern is able to double its biomass in 3–5 days and N input of 110–330 kg N/hm^2 per annum has been obtained under optimal conditions. The importance of Azolla for lowland rice production has been evaluated in numerous investigations [1–4].

After an investigation for several years, we found that Azolla is also a promising plant to be applied in controlled ecological life support system (CELSS).

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1. Biological character of Azolla

1.1. Strong photosynthetic O_2-releasing capacity

In comparison with some plants which have been proposed for use in CELSS systems, such as rice, corn and cauliflower, Azolla possesses higher photosynthetic O_2-releasing capacity (Table 1).

1.2. Rapid multiplication speed

Under suitable climatic weather, Azolla undergoes its asexual multiplication by the breakage of side branches. During vigorous growth in spring or autumn seasons, its daily multiplication rate reaches as much as 100 g/m^2; annual biomass production is 150–225 t/hm^2. Trails proved that its doubling time will be clearly shortened when grown in artificial controlled environmental condition, and biomass production increased (Table 2).

Table 1
Comparison of photosynthetic O_2-releasing capacity among some plants

Plant	Photosynthetic O_2-releasing capacity	
	mμg O_2/mg chl h [9]	mμg O_2/g plant h
Azolla	92.13	67.35
Rice	33.62	60.70
Corn	38.77	22.23
Cauliflower	64.86	49.72
Chinese chives	10.99	5.66

Note: growing at 30 °C, 8000–10 000 lux.

Table 2
Biomass production of some Azolla strains indoor culture condition

Strain	Biomass production			Calculated annual production (t/hm^2)
	Plot area (m^2)	Plot biomass (f w kg)	Growing period (d)	
A. caroniliana 3001	1.80	9.410	91	209.63
A. microphylla 4018	1.80	7.445	77	206.80
Rongping 1	1.80	10.012	91	223.01
Back-crossed Azolla MH3-1	1.80	12.667	91	282.21

Table 3
Comparison of sulfur-containing amino acid, rough protein content in *A. filiculoides* with other plants

Plant	Nutrition content	
	Met + Cys (g/100 g protein)	Protein (% dry matter)
Azolla	4.14	33
Alfalfa	3.02	20.56
Soybean	2.63	44.51
Corn	3.52	10.52

From Buckingham et al. (1978) [11].

1.3. High nutrient content

The rough protein in soybean is around 40%, as for that of Azolla is generally within 20–30%. Some of the newly bred strain contains as high as 35% rough protein, much closer to the soybean's protein content.

Azolla is rich in the amount of sulfur-containing amino acid [2], which may be of benefit to the growth of hair (Table 3).

2. Possibility of using Azolla in CELSS system

The above characters of Azolla led us to evaluate the use of Azolla in CELSS systems. We conducted the following experiments over the past several years.

2.1. Screening shade-tolerant Azolla

Generally, Azolla only needs 25–50% full sunlight for its normal growth; slight shade is of benefit to Azolla growth in field condition. That is why Chinese farmers culture Azolla in rice field at early growth stage of rice seedlings. However, when the light intensity is lower than 1500 lux, the biomass production of Azolla will be greatly decreased.

Considering the energy limitations of space stations, it is an important thing to understand the minimum light intensity by Azolla for photosynthesis. Peters (1974) found that, anaerobic acetylene reduction by *A. caroliniana* Willd was dependent on light and saturated at approximately 450 foot candles [5]; Shi [6] determined the light intensity curve during photosynthesis of *A. pinnata* and *A. filiculoides* in spring, autumn and summer seasons. The light saturation points of both Azolla species were about 6000 lux during spring, the values increased to 8000 and 14 000 lux, respectively, during summer season. The light compensation points of both species were 500–1000 lux, respectively [6].

Hence, we studied the effect of light intensity which decreased from 8300 to 6200 lux on the photosynthetic capacity of Azolla. It can be seen from the results that, when light intensity decreased down to 6200 lux, the O_2-providing efficiency was decreased by 4.4%, whereas the light intensity of 6200 lux was low for the growth

Table 4
Biomass and *in vivo* PPO activity of Azolla strains under different light intensity (8 rep.)

Strain	3200 lux (normal light)		1600 lux (mid-weak light)		800 lux (weak light)	
	Y_1 (g)	PPO_1	Y_2 (g)	PPO_2	Y_3 (g)	PPO_3
3001	3.89	13.80	3.08	15.34	2.36	16.80
90-4-6	3.50	12.28	2.75	13.83	2.32	15.48
4018	3.58	10.26	2.60	11.49	1.74	12.64
MH3-1	3.67	9.89	2.75	9.96	1.57	9.04

Note: (1) Azolla inoculum was 1.50 g for each treatment; growing duration was 7 days.
(2) $Y = 0.497 + 0.1389X$ ($Y_3 = Y$, $PPO_3 = X$), $r = 0.9560$, showing under low light conditions, the biomass production was most highly related to PPO activity in tested Azolla strains.

Table 5
Biomass production of Azolla under solution growing condition

Experiment no.	Growth period (d)	Growth area (m^2)	Inoculum (g)	Harvest (g)	Doubling time (d)
1	5	1.2	500	900	5.9
2	5	1.2	500	1100	4.4
3	5	1.2	500	1060	4.6
4	5	1.2	500	1130	4.3
$x \pm s$				1047.5 ± 102.4	4.7

of light-loving plants, but Azolla got higher photosynthetic efficiency, so it is a shade-tolerant plant. However, when light intensity decreased from 6200 lux down to 2300 lux, the O_2-providing efficiency of Azolla was greatly decreased, only 23% to compare with that under 6200 lux [7].

During our studies, we worked on breeding shade-tolerant Azolla strains by using polyphenol oxidase (PPO) activity assay; as result, some of our newly bred strains are able to grow normally under relatively low light intensity (Table 4).

These experimental results also showed that, following the decrease of light intensity, the PPO activities of Azolla strains increased. PPO activity in Azolla fronds is closely related to the shade-tolerant capacities of Azolla strains, because when an Azolla strain contains high PPO activity, it is able to resist the harm from pests and molds, and thus indirectly increases its shade-tolerant capacity [8]. It demonstrates the effectiveness of screening shade-tolerant Azolla strains by using PPO activity assay.

2.2. The growth character of Azolla is suitable to be applied in CELSS system

2.2.1. Azolla grows rapidly and occupies small space

Azolla grows while floating on the water surface. Its plant height is around 2–3 cm. So it is able to grow in multi-layer frames requiring less space compared with other plants. In addition, Azolla propagates rapidly; its doubling time under suitable environmental condition is usually 3–5 days (Table 5). It may function as an O_2-providing plant and food component simultaneously. Most of higher plants have a relatively long growth period. For example, sweet potato, which has been recommended for use in space stations, possesses the advantages that every part of the plant is edible. However, sweet potato requires large growing area, and a relatively long time duration of several months for crop maturation. Rice and wheat also require a long growing duration. Even the rapid-growing Chinese cabbage needs the duration of 20 days to become edible. So Azolla has a great advantage in this aspect.

2.2.2. Azolla is able to grow in wet-culture condition

Under space station condition, there is no water boundary layer owing to micro-gravity. It is difficult to grow Azolla on the solution. Thus a wet-culture growing method has been created after a 3-year trial. The wet-culture method is to use some special materials to make a solution-keeping pan on which Azolla stretches its roots into the substance and absorbs the nutrition from solution-keeping materials. There is no water boundary layer within solution-keeping pan and it could be used in space station condition. After resolved substance materials of wet-culture and moisture

Table 6
Biomass production of Azolla under wet-culture condition

Experiment no.	Growth period (d)	Inoculum (g)	Harvest (g)	Doubling time (d)
1	10	175	376	9.12
2	10	175	328	11.03
3	10	175	363	9.50
4	10	175	393	8.57
5	10	175	365	9.43
$x \pm s$			365 ± 23.86	9.43

recycling system in wet-culture pan, Azolla grows well in wet-culture condition although the growth speed is slightly lower than solution culture (Table 6).

It can be seen from Table 6 that when the environmental condition was optimized, the multiplication of Azolla remained high, the doubling time ranged 9–11 d, with averaged value of 9.43 days. It is suitable to apply in CELSS.

3. O_2-providing function of Azolla

When concerning the function of Azolla in CELSS system, the O_2-providing capacity of Azolla is the most important component.

The dynamic change of O_2–CO_2 concentration in "Azolla–human" ecosystem with different Azolla-culturing solutions was determined in a controlled environment closed chamber.

3.1. Azolla strain

MH3-1 is a back-crossed hybrid Azolla progeny from *A. microphylla* and *A. mexicana*.

3.2. Experimental method

In the controlled closed chamber, 10 Azolla-culturing growth chambers were installed (each Azolla-culturing container occupies $1.45\,m^2$), thus the total Azolla-culturing area was $14.5\,m^2$. A low energy-consuming light source was adopted as the source of artificial illumination. The light intensity for Azolla growth in the chamber was 6000 lux. During human life support experiments, the dynamic change of O_2–CO_2 concentration in the closed chamber, the human living and working condition and Azolla growing situation were monitored.

3.3. Monitoring systems used during the experiments

Temperature-monitoring, humidity-monitoring and O_2–CO_2 concentration-monitoring systems were installed in the closed chamber. The equipment system was running normally.

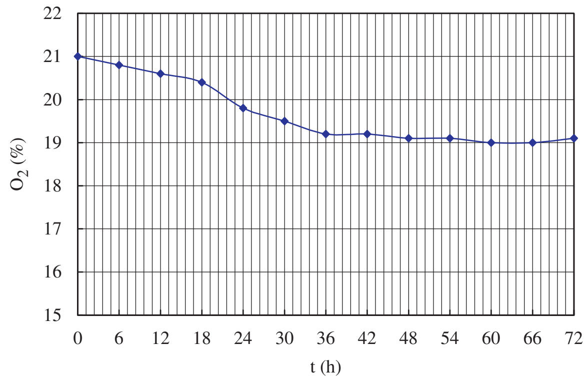

Fig. 1. O_2 concentration in the human life support test in a closed chamber during the experimental period.

3.4. Experimental results

(1) Environmental factors: The chamber capacity is $4.06 \times 2.44 \times 2.69 = 26.65\,m^3$.

Under standard temperature and air pressure, the internal atmosphere is $1.295\,kg/m^3$, among which, O_2 constitutes $1.429\,kg/m^3$ and CO_2 constitutes $1.964\,kg/m^3$. O_2 comprises 21% of atmospheric total volume. CO_2 occupies 0.03%.

(2) O_2-providing experiment by Azolla: The O_2-providing experiment by Azolla in the controlled closed chamber showed that Azolla plants with $16\,m^2$ growing area were able to provide the O_2 used by two persons. The O_2 level was maintained at 19% between 54 and 72 h of the experiment duration (Fig. 1).

4. Azolla used as an important biological part for the purification of urine

The amount of water required in a long-term space-flight is large. In order to reduce the burden of water supply from Earth, the space station needs to resolve the problem of water supply and sewage water treatment.

For this reason, the recovery and regeneration of the urine solution of spaceflight crew and then its further utilization possess a key importance. Many investigations on this aspect have been reported. However, the recovered water from urine was mostly using physical and chemical methods; its reuse poses a psychological problem for the crew. Our method adopted a comprehensive urine treatment with biological purification—UV photocatalytic oxidation. The urine solution can be not only used as the nutrient solution for biological elements (plants) in spacecraft, but also can be regenerated through transpiration into drinking water.

4.1. Selection of biological species

The selection of biological species is the key technique for urine purification. The criteria for this selection were:

- possesses a strong capacity to absorb NH_4–N and concentrated ions;
- rapid propagation, can meet the requirement for continuous urine purification;
- possesses an edible value for life-supporting system in space station;
- strong O_2-releasing capacity, can be used as a supplementary oxygen provider.

4.2. Screening Azolla as biological part

According to the above mentioned criteria, we have undertaken a strict selection from many kinds of plants, and finally chosen Azolla because of the following reasons:

- Azolla possesses a strong capacity to absorb NH_4–N. Trial showed that, after purification and absorption for 5 days by Azolla, NH_4–N in the urine solution was reduced from 25.25 to 1.80 mg/L, meaning the NH_4–N was reduced by 92.9% (Table 7 and Fig. 2).
- Azolla has a strong ion-enriching capacity from its surrounding solution. Experiments showed that Azolla is able to concentrate trace potassium from irrigation water. Its absorbing peak occurs in an ion concentration of 0.85 ppm, whereas the absorbing peak of rice plants occurs in 8 ppm. Azolla was nearly 10 times more effective than rice plants.

According to the experiment conducted by Applied Ecological Institute of the Chinese Academy of Sciences, when growing Azolla in sewage water, Ge content in Azolla fronds was 5.48 ppm and 59.02% higher than the Azolla in clean water solution, Pb was 81.1 ppm and 172.67% higher, Zn was 7.134 ppm and 200.38% higher than Ck treatment (in clean water). This illustrates that Azolla is able to remove heavy metal ions from its surrounding solution.

- Azolla multiplies rapidly. This has been discussed in Section 1.2.
- Azolla contains about 30% rough protein (by dry weight) and is an edible plant. After processing, it can be used as salad or stuffing for dumplings.
- Azolla has strong O_2-releasing capacity.

Table 7
The variation of NH_4–N in human urine solution after taking up by Azolla

Azolla growing period (d)	0	1	2	3	4	5
NH_4–N content (mg/L)	25.25	18.04	12.03	8.42	5.41	1.80

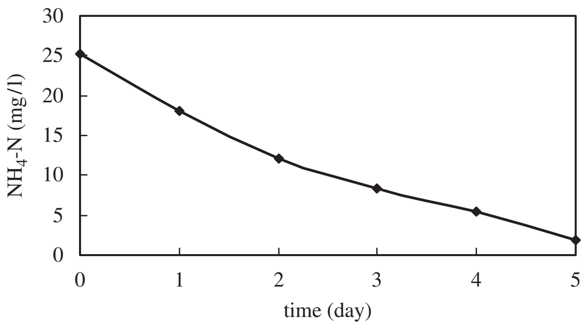

Fig. 2. The variation of NH_4–N in human urine solution after absorption by Azolla.

4.3. Purification of urine solution

- After purifying and absorbing by Azolla, the bacteriological indexes in urine solution had less variation, but the indexes of chroma, feculent degree and N content were strongly decreased (Table 8), and reached the goal of preliminary purification.
- After preliminary biological purification, the urine solution is passed through the poly-pore filter in filtrating column to remove bad smell, and then the urine solution comes into a photocatalytic-oxidation reactor. After that, the urine solution was treated and reaches drinking water standards (GB5749-85) issued by the Chinese national government.

Table 8
Bacteriological indexes, chroma, feculent degree and N content in urine solution after absorption of Azolla

Treatment	Chroma	Feculent degree (NTU)	Smell	P54H	Urea (mg/L)	Nitrate (mg/L)	Nitrite (mg/L)	Total bacteria (cfu/mL)	Coliform group (no./L)
Urine solution (Ck)	150	11.0	Stench	7.3	2.0	0.8	0.3	2.1×10^3	230
Urine solution (growing Azolla)	21	6.0	Some stench	7.2	1.6	0.6	0.1	1.9×10^3	210

Table 9
Tested result of harmful heavy metal ions contained in Azolla biomass (Fujian Inspection and Testing Centre for Agricultural Product Quality and Safety)

Test result	Test items	Value
	As, mg/kg (product)	Did not find out (limit: 0.001)
	Hg, mg/kg (product)	Did not find out (limit: 0.001)
	Pb, mg/kg (product)	0.095
	Cu, mg/kg (product)	5.000

Note: Azolla grows on IRRI Azolla culture solution [10].

5. Azolla can be served as food

Azolla grown in a pollution-free environment contains less heavy metal ions (Table 9), and can be processed into salad, cold dishes and frying food after the fronds are sterilized by steam. It also can be served as part of main dishes, such as Chinese spring rolls or dumplings in which Azolla can account for as much as 60% by weight.

Feeding Azolla to some small-size animals, then using these animals for human food is another way of using Azolla as a food source. For example, by using Azolla to raise snails, earthworms and fish, Azolla can become an important part of a new type of food chain.

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