

Nutrient Balance and Nitrogen Cycling In a Multistage, Multispecies Space Farm

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The ability to recycle biomass and waste water into clean water and food is a core technology set for permanent human settlement of space. A space farm system is one way of performing the recycling task, while providing caloric energy and nutrients to the colonists. Previous papers have focused on the development of four non-human stages: a Yeast-Bacteria Reactor Stage, an Algae Rector Stage, a Hydroponic Stage, and an Aquatic Stage. Previous work also used a single species set for the Hydroponic and Aquatic Stages and manual balancing for a single year of water, biomass, carbon dioxide, and oxygen. Mass balance was the driving method, regardless of biomass composition. This work did indeed show a mass balance was possible for the species (carp, tomatoes) chosen. Further work since has developed software to allow any number of species given data, for any period of time, for the Hydroponic and Aquatic Stages. This further work illuminated the need for a more detailed examination of the nutrient components produced for both the human colonists in the habitat and the animals in the Aquatic Stage. Nitrogen cycling is a key component in protein production and digestion, and tomatoes are not nitrogen fixing. Further, the types of carbohydrates and proteins provided by crops to yeast, animals, or people is critical to the farm cycle. An enhanced modeling program was developed to examine this balance, and graphs the components over time for each stage and species, and further analysis illustrated the need to incorporate a more diverse species composition in both plants and animals. This analysis resulted in a series of possible plants, including legumes and grains, and for dietary diversity included other aquatic species. This paper will show the now detailed nutrient balance and species trade-offs for a four stage model with multiple species in each stage, and the ensuing nutrition, initial resource requirements, resupply requirements, and space requirements.

Nomenclature

Primary Mass Components (Capitalized for a whole stage. lowercase for per capita values.):

B_i	=	dry biomass input to living biomass
B_o	=	dry biomass output to living biomass
$B_{o,g}$	=	dry biomass by living mass output as living biomass (grown)
$B_{o,g,inedible}$	=	dry biomass by living mass output as living biomass (grown) that is inedible to humans.
$B_{o,g,edible}$	=	dry biomass by living mass output as living biomass (grown) that is edible to humans (i.e. crop)
$B_{o,e}$	=	dry biomass by living mass output as excretions
C_i	=	carbon dioxide (CO ₂) input by living mass
C_o	=	carbon dioxide (CO ₂) output by living mass
O_i	=	oxygen gas (O ₂) input by living mass
O_o	=	oxygen gas (O ₂) output by living mass
W_i	=	water (H ₂ O) input by living mass
W_o	=	water (H ₂ O) output by living mass

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I. Introduction

The ability to recycle biomass and waste water into clean water and food is a core technology set for permanent human settlement of space. A space farm system is one way of performing the recycling task, while providing caloric energy and nutrients to the colonists. Previous papers¹ have focused on the development of four non-human stage types: a Yeast-Bacteria Reactor Stage, an Algae Rector Stage, a Hydroponic Stage, and an Aquatic Stage. Previous work also used a single species set for the Hydroponic (Tomatoes), Aquatic Stage (Silver Carp), Algae Reactor Stage (*Chlorophyta*), and manual balancing for a single year of water, biomass, carbon dioxide, and oxygen. Mass balance was the driving method, regardless of biomass composition. This earlier work did indeed show a mass balance was possible for the species chosen, though at a very coarse level. The manual approach had scalability limits, since it was based on copy and paste into a spreadsheet. This limit showed the need to create a simulation model to get closer to reality. We developed software in PERL and XML, leading to an initial prototype, then later refined that prototype to allow any number of species given data, for any period of time, focusing on the detailed cycling of biomass and especially nitrogen. The key was removing population as individuals, and replacing population in terms of living mass, for every stage type but the habitat. This further work illuminated the need for a more detailed examination of the nutrient components produced for both the human colonists in the habitat and the animals in the Aquatic Stage. Nitrogen cycling is a key component in protein production and digestion, and tomatoes are not as nitrogen fixing as other crops, nor are tomatoes high in protein. Further, the types of carbohydrates and proteins provided by crops to yeast, animals, or people is critical to the farm cycle. Fiber (cellulose) is an accumulator of carbon, and is produced by plants, passed through by animals and people. This analysis illustrated the need to incorporate a more diverse species composition in both plants and animals, and tracking for key nutritional components: fiber, (edible) carbohydrates, fats, excreted (nitrogen containing), and protein (nitrogen containing). The nitrogen element composition was found from biomass inputs and outputs for each species in each stage, using assessments by amino acid formulas and content in the stage, and modeled as it passed through the organisms.

This paper will show the now detailed nutrient balance and species trade-offs for a four stage-type model with multiple species in each stage type, and the ensuing nutrition, initial resource requirements, resupply requirements, and space requirements.

II. Approach

A. Mass by Element and Chemistry

As in prior work, each stage is a mass balance. Each stage, an aggregate of individual organisms considered as a mass, takes in, and either excreted, or becomes a crop, of gasses, solids, and liquids, considered together, and categorized by oxygen, carbon dioxide, water, and biomass. 'Biomass' here means anything that is not oxygen gas, water, or carbon dioxide. In this work, biomass is subdivided by composition, including tracking nitrogen, and nutrition components. It is assumed that mass between stages is ideally distributed to the other stages and the human habitat. Given limitless energy, gas and liquid forms of water are freely converted, and gasses can be perfectly infused and extracted from liquids. The human habitat interacts by consuming crop items, oxygen, and water, and excreting water, biomass, and carbon dioxide. Mass is then balanced between what is consumed, what is excreted, and what is absorbed as growth and then harvested later. Beginning with Eq.14 from Ref. 1, we have:

$$b_i + w_i + o_i + c_i = b_o + w_o + o_o + c_o \text{ averaged per population element.} \quad (1)$$

$$B_i + W_i + O_i + C_i = B_o + W_o + O_o + C_o \text{ for the whole stage.} \quad (2)$$

where outputs include a continuous harvest replaced continuously by growth. Continuity can be accomplished for periodic items by staggering planting and altering hormones, lighting, and water.

It can also be seen that as mass is conserved, so are elements in normal chemical reactions. The farm features cycling of each of the core biological elements, carbon, oxygen, hydrogen, sulfur, and nitrogen. Due to the importance of nitrogen in proteins for growth, it is of key importance. Salts, minerals, and sulfur are tracked as ash included in biomass. Tracking these elements in photosynthesis, aerobic respiration, and anaerobic respiration

becomes important for each stage in the farm. The following table shows many of the core compounds in the farm, and what they contain for nitrogen, carbon, hydrogen, sulfur, and oxygen (N, C, H, S, O respectively):

Table 1. Common biological compounds^{1,2,5,6}

Name	Formula	Molecular Weight ^{ref}	Atoms in Formula					Relative mass by Element				
			C	O	H	N	S	C	O	H	N	S
Elemental Carbon	C	12.01	1					100%	0%	0%	0%	0%
Elemental Oxygen	O	16		1				0%	100%	0%	0%	0%
Elemental Hydrogen	H	1.01			1			0%	0%	100%	0%	0%
Elemental Nitrogen	N	14.01				1		0%	0%	0%	100%	0%
Elemental Sulfur	S	32.06					1	0%	0%	0%	0%	100%
Carbon Dioxide	CO2	44.01	1	2				27%	73%	0%	0%	0%
Oxygen gas	O2	32		2				0%	100%	0%	0%	0%
Water	H2O	18.01		1	2			0%	89%	11%	0%	0%
Fructose (and most dietary Carbohydrates once hydrated)	C6H12O6	180.15	6	6	12			40%	53%	7%	0%	0%
Cellulose	C6H10O5	162.14	6	5	10			44%	49%	6%	0%	0%
Starch (i.e. Chains of unhydrated monosaccharides)	(C6H10O5) _n	162.14 x n	6	5	10			44%	49%	6%	0%	0%
Ethanol	C2H5OH	46.07						52%	35%	13%	0%	0%
Cholesterol (a fat)	C27H46O	386.66	27	1	46			84%	4%	12%	0%	0%
Fat Triglyceride (most fats)	C55H98O6	855.37	55	6	98			77%	11%	12%	0%	0%
Methane	CH3	15.03	1		3			80%	0%	20%	0%	0%
Urea	CH4N2O	60.06	1	1	4	2		20%	27%	7%	47%	0%
Ammonia	NH3	17.03			3	1		0%	0%	18%	82%	0%
Ammonium	NH4+	18.04			4	1		0%	0%	22%	78%	0%
Alanine	C3H7NO2	89.09	3	2	7	1		40%	36%	8%	16%	0%
Arginine	C6H14N4O2	174.2	6	2	14	4		41%	18%	8%	32%	0%
Asparagine	C4H8N2O3	132.12	4	3	8	2		36%	36%	6%	21%	0%
Aspartic acid	C4H7NO4	133.1	4	4	7	1		36%	48%	5%	11%	0%
Cysteine	C3H7NO2S	121.15	3	2	7	1	1	30%	26%	6%	12%	26%
Glutamic acid	C5H9NO4	147.13	5	4	9	1		41%	43%	6%	10%	0%
Glutamine	C5H10N2O3	146.15	5	3	10	2		41%	33%	7%	19%	0%
Glycine	C2H5NO2	75.07	2	2	5	1		32%	43%	7%	19%	0%
Histidine	C6H9N3O2	155.16	6	2	9	3		46%	21%	6%	27%	0%
isoleucine	C6H13NO2	131.17	6	2	13	1		55%	24%	10%	11%	0%
Leucine	C6H13NO2	131.18	6	2	13	1		55%	24%	10%	11%	0%
lycine	C6H14N2O2	146.19	6	2	14	2		49%	22%	10%	19%	0%
Methionine	C5H11NO2S	149.21	5	2	11	1	1	40%	21%	7%	9%	21%
Phenylalanine	C9H11NO2	165.19	9	2	11	1		65%	19%	7%	8%	0%
Proline	C5H9NO2	115.13	5	2	9	1		52%	28%	8%	12%	0%
Serine	C3H7NO3	105.09	3	3	7	1		34%	46%	7%	13%	0%
Threonine	C4H9NO3	119.12	4	3	9	1		40%	40%	8%	12%	0%
Tryptophan	C11H12N2O2	204.23	11	2	12	2		65%	16%	6%	14%	0%
Tyrosine	C9H11NO3	181.19	9	3	11	1		60%	26%	6%	8%	0%
Valine	C5H11NO2	117.15	5	2	11	1		51%	27%	9%	12%	0%
Amino Acid Average			5.35	2.45	9.85	1.45	1	46%	30%	7%	15%	2%

Proteins are shapes of amino acids. It can be seen from the table that nitrogen is available from the amino acids (from proteins), and urea and ammonia, or the ionic ammonium. Urea breaks down using water to ammonium (or ammonia) and carbon dioxide. Tracking these elements and chemicals in photosynthesis, aerobic respiration, and anaerobic respiration becomes important for each stage in the farm.

Table 2. Typical Carbon – Oxygen - Hydrogen Pathways and Ratios

Created via Pathway	Decomposed via Pathway	Product	by Mass Relative to Product Mass(=input, + = output)				
			CO2	H2O	O2	Nitrogen	Sulfur
Fiber via Photosynthesis: 6CO2+5H2O → C6H10O5 + 6O2	Enzymatic, Aerobic: C6H10O5 + 6O2 --> 6CO2+5H2O	C6H10O5	-1.63	-0.56	1.18	0	0
Fat via photosynthesis via enzyme core reaction 55CO2+49H2O --> C55H98O6 + 76.5O2	Aerobic via enzyme C55H98O6 + 76.5O2 --> 55CO2+49H2O	C55H98O6xn	-2.83	-1.03	2.86	0	0
Carbohydrate via Photosynthesis 6CO2+6H2O → C6H12O6 + 6O2	Carbohydrate Aerobic C6H12O6 + 6O2 --> 6CO2+6H2O	C6H12O6	-1.47	-0.60	1.07	0	0

In photosynthetic organisms, such as those in the Hydroponic Stages, and Algae Reactor Stages, carbons come from carbon dioxide, nitrogen from either the air (as on Earth) or from consumed biomass (as for Earth farms, and especially for the space farm) in the form of urea, ammonia, oxygen from carbon dioxide and water, and hydrogen largely from water, some from ammonia or urea. Plants make mostly cellulose, some starches, some other sugars (such as fructose), some proteins, and some fats. Oxygen gas is a product of photosynthesis, taken from the reactions to make sugars from water and carbon dioxide. Amino acids which are made into proteins are assembled using the nitrogen from ammonia and other consumed biomass. Water is used in photosynthesis and many other reactions, but also essentially inflates living biomass. The primary path for carbon is in the carbon dioxide to sugar process. The primary path for nitrogen are the ammonia to amino acid processes. This exchange can be seen below:

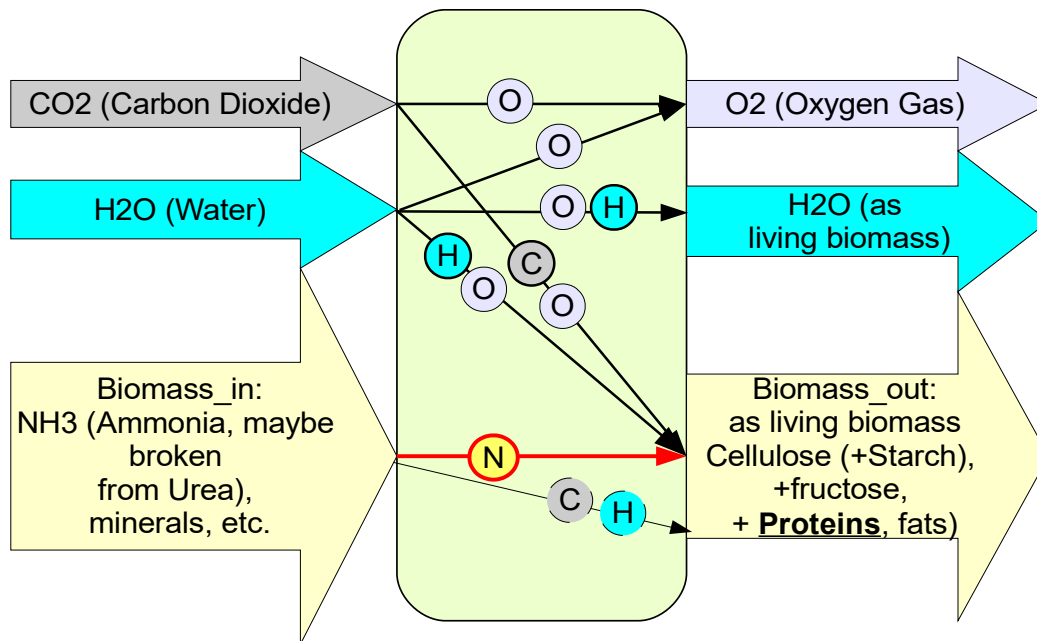


Figure 1. Element movement in photosynthetic organisms. Note : Transpiration (not depicted) in vascular plants is caputed, condensed, and recycled inside the stage, not affecting mass balance.

A similar diagram can be made for largely aerobic organisms, such as animals and fungi and bacteria (i.e. the Yeast-Bacteria Reactor operating in aerobic mode). Oxygen gas is consumed, with water, and biomass in the form of carbohydrates, lipids, and proteins, salts, and reactions result in carbon dioxide, water, and biomass in the form of proteins, fats, urea, ammonia, methane and other compounds. Urea (which can be broken into ammonia or ammonium) is produced by the breakdown of proteins, and is excreted with water. Carbon dioxide is exhaled, protein and fats become living biomass inflated by water, though some proteins and fats are also passed. Dietary fiber, i.e. cellulose from plant foods, is passed through as well, unless processed (by bacteria in some mammals for example, or the Yeast-Bacteria Reactor). The primary path for carbons is the breakdown of sugars from biomass to carbon dioxide. The primary paths for nitrogen are the assembly of proteins from amino acids, and the breakdown of amino acids from proteins into urea (via ammonia). This exchange is similar to the diagram above and results in the diagram below.

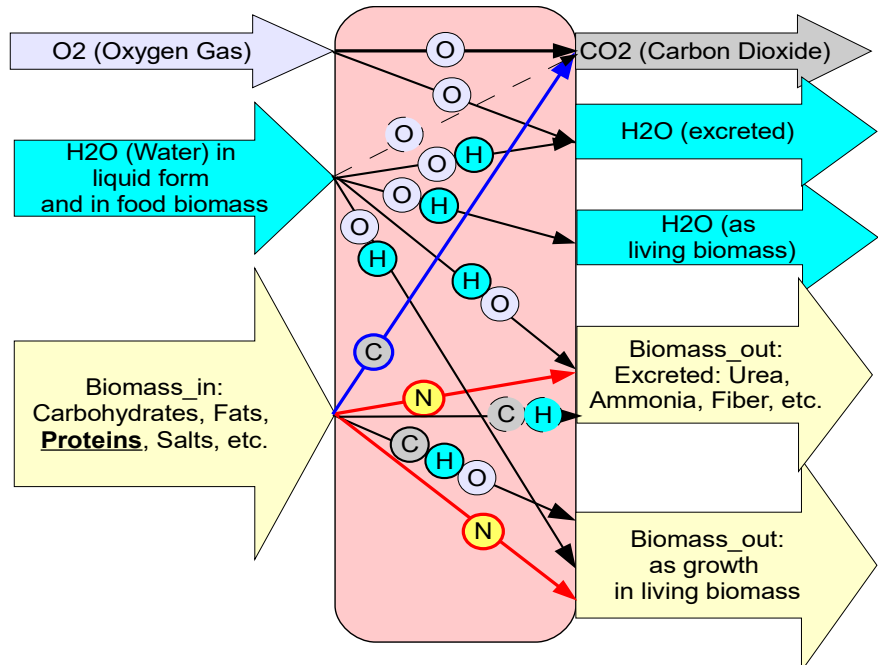


Figure 2. Element movement in aerobic organisms.

Anaerobic processes as for some modes for yeast and bacteria (and in animals, including people, under stress), are a chopped form of the aerobic processes above. In the absence of oxygen, organisms produce alcohols from water and sugars, excreted as biomass out, and produce proteins and fats in living tissues (living biomass out, inflated with water). This can be seen in the next figure.

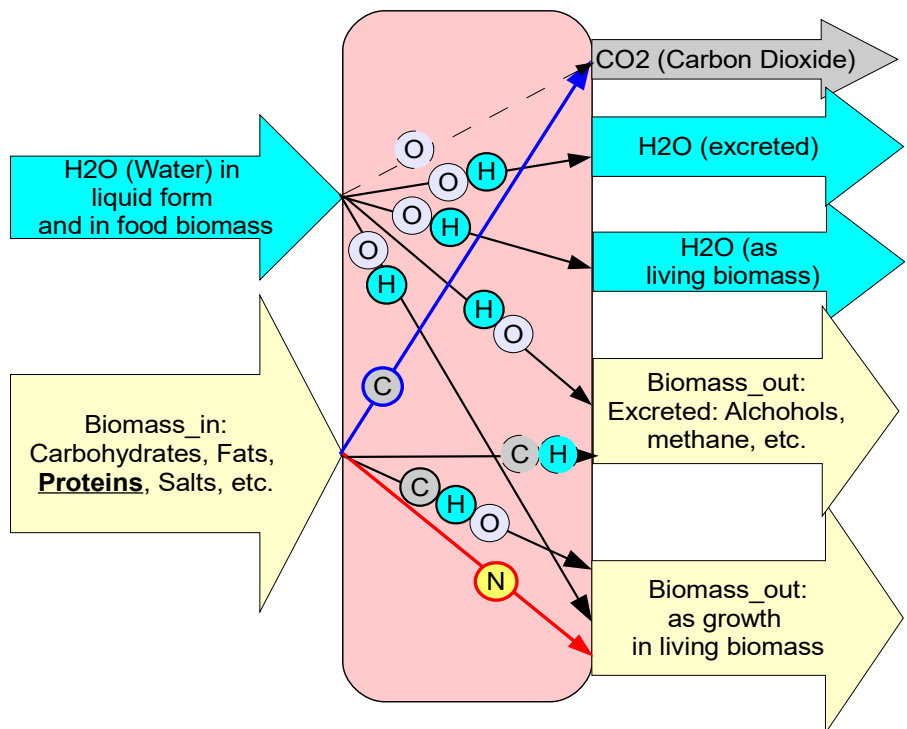


Figure 3. Element movement in anaerobic organisms/cells.

Overall for all organisms in the farm, outputs are a sum then of excretes, in oxygen, carbon dioxide, water, and biomass (for plants), and of crop items consumable (living biomass out) by the humans in the habitat or inedible or edible biomass by animals, and yeast + bacteria.

Table 3. Inputs and outputs to each stage type.

Stage Type	Example Organisms	Inputs	Outputs
Habitat	People	Oxygen, Water, Biomass_in (Food: Proteins, Fats, Carbohydrates, Fiber and Kcal)	Carbon Dioxide, Water (Excrete), biomass_out (excreted):Fiber, Urea, other waste from food.
Algae Reactor	Chlorophyta, Spirulina, Kelp	Carbon Dioxide, Water, Biomass_in(Ammonia/um, other minerals)	Oxygen , Water (in growth), Biomass_out (fiber, carbohydrates, proteins, fats)
Yeast-Bacteria Reactor	Yeasts, Bacteria	Variable: Aerobic or Anerobic	Variable: Aerobic or Anerobic
Hydroponic	Tomato, Rice, Soybeans	Carbon Dioxide, Water, Biomass_in(Ammonia/um, other minerals)	Oxygen, Water (in growth), Biomass_out (inedible and crop fiber): carbohydrates, proteins, fats
Aquatic	Carp, Tilapia, Shrimp, Krill	Oxygen, Water, Biomass_in (Food: Proteins, Fats, Carbohydrates, Fiber and Kcal)	Carbon Dioxide, Water (Excrete and in growth), biomass_out (excreted):Fiber, Urea, other waste from food. (Growth/Crop): Protein, Fats, Carbohydrates

In reality biomass must be in the right form to be consumable by the organisms in other stages. Carp can't eat ammonia from animal excretes (it can become toxic to them). Carp and shrimp can eat portions of plants inedible to people but the fiber is largely passed through. Photosynthetic organisms require nitrogen in simple forms (i.e. ammonia, nitrates, etc. as from excretes) to grow, then convert these to more complex amino acids and proteins. This results in a flow between stages as in Fig. 4.

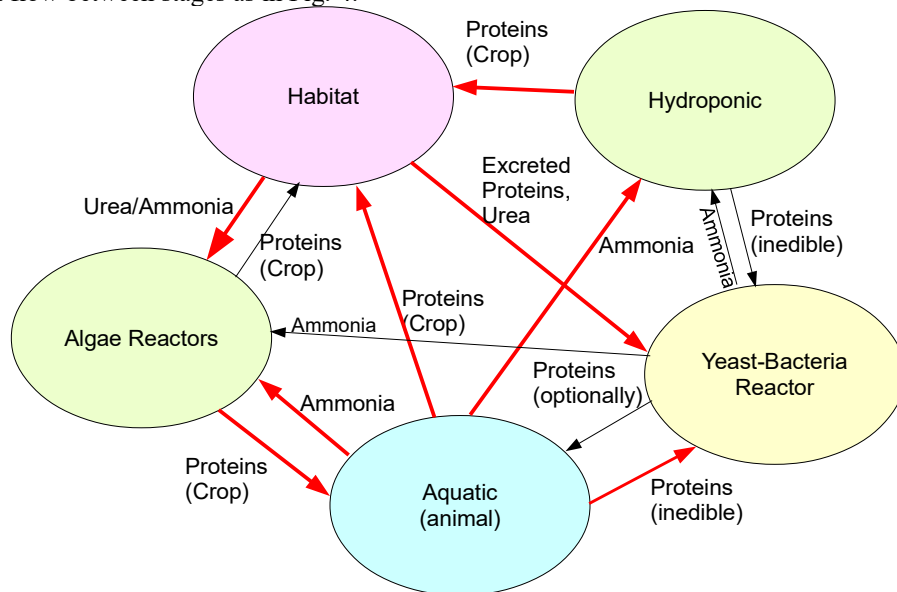


Figure 4. Nitrogen flow between stage types.

Further people require nutrients in certain forms to be healthy, derived from molecular masses, NASA guidance³, and US Recommended Daily Allowance for a 2,000 kcal diet⁴ as noted roughly below (note C, O, H, N are elemental):

Table 4. Human stage inputs per person.

Human inputs per person		% by mass				kg mass			
Nutrient (Dry Biomass in)	kg/person/day	C	O	H	N	C	O	H	N
Lipids+Cholesterol	0.0703	83.87%	4.14%	11.99%	0.00%	0.059	0.003	0.008	0.000
Carbohydrates	0.3240	42.11%	51.41%	6.48%	0.00%	0.136	0.167	0.021	0.000
Cellulose (Fiber)	0.0250	44.45%	49.34%	6.22%	0.00%	0.011	0.012	0.002	0.000
Proteins	0.0500	45.28%	30.11%	7.20%	14.88%	0.023	0.015	0.004	0.007
NET Oxygen in**	0.5900	0	100.00%	0	0	0.000	0.590	0.000	0.000
Water in*	5.0000	0.00%	88.81%	11.19%	0.00%	0.000	4.441	0.559	0.000
NET INPUT	6.059					0.229	5.227	0.594	0.007
USABLE INPUT (i.e. Input -cellulose)	6.034					0.22	5.22	0.59	0.01
Excrete (Dry Biomass out)	0.034					0.008	0.007	0.011	0.007
Carbon Dioxide	0.811	27.29%	72.71%	0.00%	0.00%	0.221	0.590	0.000	0.000
Water out	5.214	0.00%	88.81%	11.19%	0.00%	0.000	4.631	0.583	0.000
NET OUTPUT	6.059					0.229	5.227	0.594	0.007

* = Includes water in wet biomass from foods, assumes 3 liters drink, and 2 liters in food
 ** = NET Oxygen in is Oxygen inhaled – minus Oxygen exhaled

In Table 4, the carbons in carbon dioxide comes from digestion of lipids, carbohydrates, and proteins, and assumes no absorption for growth. Oxygen is shown as a net of inhalation and exhalation, since in the stage oxygen and other gasses are managed and only to overall needs of the stage drive inputs to the habitat and the needs drive the outputs. The sum of excreted biomass and water out are the black water sent into the farm, carbon dioxide pooled as a gas into the farm for gas management by a central controller and system of tanks. Those who have read the prior paper¹ will note the lower water requirement in Table 4. This is due to a better understanding of needs, and due to the assumption that gray water is recycled in the habitat itself. It is key from the table to note that the only source of nitrogen is from proteins, which include amino acids. This nitrogen is in turn excreted in sweat, feces, and urine as both proteins and urea among other compounds.

In order to feed the now nitrogen sensitive simulator, a series of similar calculations are needed for each organism in the space farm. Unlike in the habitat, the remaining space farm as noted previously, uses living mass per kilogram in lieu of population.

Since all Hydroponic and Aquatic stages have crops, harvested continuously, biomass is subdivided among grown and excreted components:

$$B_o = B_{o,g} + B_{o,e} \quad \text{for the whole stage.} \quad (3)$$

$$B_{o,g} = B_{o,g,inedible} + B_{o,g,edible} \quad \text{for the whole stage.} \quad (4)$$

Many growth components of $B_{o,g}$ include biomass that is edible, $B_{o,g,edible}$, and not human consumable, i.e. inedible, $B_{o,g,inedible}$, that must be digested in the Yeast-Bacteria Reactor, or fed to another stage that can digest the non-consumable parts. In the case of excreted, $B_{o,e}$, some are consumed in other stages as urea from humans can be consumed by hydroponic crops or algae. As a result, tracking both the human consumable portions, and the remainder is also important. For hydroponic crops, the non-consumable parts will be trimmings from roots, leaves, and stems in fruiting crops (including many grains and legumes), or remaining roots, leaves, and stems for tubers, or just the roots for fiber crops (such as lettuce). These portions in many cases can be fed to animals after grinding, or ground and fed to the Yeast-Bacterial Reactor. For Aquatic animal crops, few portions are non-consumable in one

form or another (example fish meal from the non-fillet portions), so it is assumed the whole mass is edible. In this paper I also examined plant aquatic species, essentially alternate Algae Reactors, such as *Spirulina* and kelp, which can be completely consumed. Fortunately for many species, the United States Department of Agriculture (USDA) has loaded amino acid and other nutrients into their USDA Food Composition Databases⁷, which proved very useful for the edible portions of nearly all crops, and allowed direct calculation of element content with using the amino acid composition.

In the case where harvest is continuous (as assumed in this paper), nitrogen in must be the same as nitrogen out, and not accumulated after steady state. This doesn't mean the nitrogen is available to the habitat, due to the transfer of nitrogen to inedible portions. It is assumed that processing using mechanical means might make the inedible portions feed for animals or the Yeast-Bacteria Reactor.

All reactions are assumed to follow mass-balance, and each reaction is represented in ratios of the inputs in Eq. 3.

For hydroponic plants, finding the inedible ratio to edible ratio in growth biomass out, required both a harvest index (which is harvest biomass to edible biomass) and ratio of root to harvest for grains and above surface crops. For tubers, the entire plant is typically taken, reducing the need for root calculation, while for edible leaves, the whole plant is harvested, and nearly all edible. Accounting for nitrogen in dry masses in biomass in and biomass out therefore is finding both the nutrient values for the crop, and nitrogen content in the inedible portions. Hydroponic crop stages are assumed to use all biomass excretes provided, so these are set to 0 in output. Outputs and inputs for crop species started with the USDA Nutrition Data then located a reference for growth rate to get mass per day per living mass. For plants the division of mass between inedible portions (stem, leaves, and root for grains and fruits) and location of nitrogen was found. Algae Reactor crops were assumed to be entirely edible. An allocation of nitrogen, carbon, oxygen, hydrogen, and sulfur, for all components of the plant was computed (kg per living kg per day) using data as in Table 1 above. A mass balance for inputs was then computed assuming carbon dioxide was the primary source of carbon, and biomass inputs the source of nitrogen and sulfur and ash. Water input was assumed the source for hydrogen in output, and added to moisture from biomass outputs. Oxygen computed from the excess from input metabolic water and carbon dioxide.

Algae Reactor inputs and outputs were computed similar to hydroponic plants, except that the harvest is a regular mass of algae cells, all assumed edible *Spirulina* and kelp are reactor crops, as is *Chlorophyta*.

Animal (aquatic) crops also need to consume nitrogen, in the form of proteins including component amino acids, along with fiber, carbohydrates, and lipids. These crops are raised in raceways or tanks, with re-circulation pumps, coarse filters (which feed the Yeast-Bacteria Reactor), and lighting, in ideally controlled temperature and chemistry. Like the human habitat, biomass in represents a composition of elements, and biomass out includes excretes and crops. Excretes will contain a ratio of consumed nitrogen, as will tissues consumed as crops. Animals consume more food than they use, and feeds containing nitrogen are fed (overfed) at a ratio of the nitrogen needed, along with compounds that can be broken into sugars for energy. As for photosynthetic organisms, animal data began with the USDA Nutrition Data, and a computation of nitrogen, carbon, oxygen, hydrogen, and sulfur, then assuming oxygen was primarily from oxygen gas inputs, nitrogen from biomass in. Amino acid content in the edible portions (i.e. the whole animal here) was used to compute nitrogen if available, otherwise the protein average was used. References were found to determine the growth ratio of living biomass to energy. Energy consumption drives oxygen consumption, and outputs of carbon dioxide and a small amount of water. Nutrient content of the resultant crop was used with the to find feed amount, then combined with the estimated sugars needed for energy implied by oxygen consumption. As for photosynthetic crops, sulfur from amino acids in proteins, was added to ash to add to feed (biomass in) inputs.

Table 5. List of Farm Species, References, and Assumptions.

SPECIES	Scientific Name	Dietary Source	Metabolic Sources	Assumptions
Rice	<i>Oryza sp. (hybrids)</i>	USDA NDD #20088 ⁷	8,9,10,11,12	Efficiency is equal or greater than field production, entire plant is harvested, including roots. Planting and growth is staggered for continuous production
Tomato	<i>Solanum lycopersicum (hybrids)</i>	USDA NDD #11529 ⁷	1,24,25,26	Plants are picked for fruit, and trimmed to stay the same size continuously
Soybeans	<i>Glycine max (hybrids)</i>	USDA NDD #11450 ⁷	13,14,15	Efficiency is equal or greater than field production, entire plant is harvested, including roots. Planting and growth is staggered for continuous production
Chlorophyta	<i>Chlorophyta sp.</i>	Ref 30, Nutrition facts, compared to Ref 29	28,29,30,31,32	Doubled biomass is consumed as edible biomass by humans or animals
Spirulina	<i>Spirulina sp.</i>	USDA NDD #11666 ⁷	22	Doubled biomass is consumed as edible biomass by humans or animals
Kelp	<i>Macrocystis sp.</i>	USDA NDD #11445 ⁷	21	All plant is edible. Growth is continuously trimmed to provide edible biomass
Silver Carp	<i>Hypophthalmichthys molitrix</i>	USDA NDD #15008 ⁷	16	Entire mature organism is consumed. Breeders and small juveniles are a very small mass relative to crop. Crop is staggered to allow continuous harvest and replacement.
Tilapia	<i>Oreochromis sp.</i>	USDA NDD #15261 ⁷	17,18	Entire mature organism is consumed. Breeders and small juveniles are a very small mass relative to crop. Crop is staggered to allow continuous harvest and replacement.
Shrimp	<i>Litopenaeus sp. Or Macrobrachum sp.</i>	USDA NDD #15270 ⁷	19,20,21	Entire mature organism is consumed. Breeders and small juveniles are a very small mass relative to crop. Crop is staggered to allow continuous harvest and replacement. Growth is at least as good as pond rearing.
Yeast-Bacteria Reactor	Many species on film and in tanks	USDA NDD #18375 ⁷	27	Excretes produced only from protein aerobic or anerobic respiration, edible biomass only produced as needed if the system is lacking biomass

For all crop species examined, the following tables describe the mass balance and nitrogen-biomass budgets.

Table 6. Mass Balance for farm species other than the Yeast-Bacteria Reactor

	kg per kg live mass per day productive							
	Inputs				Outputs			
Crop	CO2	H2O	O2	Biomass_in (total)	CO2	H2O	O2	Biomass out (total)
Rice	0.01214	0.00507	0	0.00024	0	0.00062	0.00908	0.00776
Tomato	0.02349	0.140137	0	0.00156	0	0.13052	0.01923	0.01543
Soybeans	0.01841	0.020902	0	0.00243	0	0.01397	0.01090	0.01688
Chlorophyta	0.01557	0.83397	0	0.00176	0	0.82800	0.01409	0.00920
Spirulina	0.00873	0.05260	0	0.00075	0	0.05206	0.00443	0.00559
Kelp	0.00398	0.017634	0	0.00136	0	0.01614	0.00298	0.00386
Silver Carp	0	0.002079	0.003840	0.00446	0.005281	0.00424	0	0.00086
Tilapia	0	0.004723	0.001466	0.10000	0.002016	0.00474	0	0.09944
Shrimp	0	0.006519	0.009600	0.01506	0.013204	0.00652	0	0.01145

Table 7. Biomass and nitrogen budgets by species.

Crop	Mass (kg) dry biomass per kg live mass per day productive							
	TOTAL				Nitrogen			
	Biomass in	Biomass out excrete	Biomass out growth edible	Biomass out growth inedible	Biomass in	Biomass out excrete	Biomass out growth edible	Biomass out growth inedible
Rice	0.00024	0	0.003374	0.004387	0.000111	0	0.00006898	0.0000417
Tomato	0.00156	0	0.004800	0.01063	0.000614	0	0.00007353	0.0005403
Soybeans	0.00243	0	0.003780	0.01310	0.000794	0	0.00006896	0.0007251
Chlorophyta	0.00176	0	0.009200	0	0.000610	0	0.0006099	0
Spirulina	0.00075106	0	0.005589	0	0.000494	0	0.0004944	0
Kelp	0.00136	0	0.003861	0	0.000042	0	0.0000418	0
Silver Carp	0.00446	0.000198	0.000661	0	0.000089	0.0000205	0.000068	0
Tilapia	0.10000	0.098061	0.001375	0	0.004319	0.0041445	0.000175	0
Shrimp	0.01506	0.009639	0.001815	0	0.000820	0.0005741	0.000246	0

The Yeast-Bacteria Reactor is a combination of biochemical reactors, mechanical tanks and mixers, and control systems, that use tailored yeasts and bacteria cultures, possibly film mediated, to keep the chemical balance of the system. It was assumed to operate in anaerobic and aerobic modes as required to balance mass, and to breakdown fiber and fat to carbohydrate or other biomass types as required. This stage was assumed to be able to process 50% of its living mass per day, which is similar to the doubling rate of bacteria and bread yeasts. Inedible biomass can end up here or in Aquatic Stages as feed. It is very important that the reactor can break down and use cellulose (i.e. fiber), or else all the carbon in the system would eventually accumulate as indigestible fiber.

B. Seeking Balance

Given the number of species, an iterative approach was used. The primary criterion were balancing water, carbon dioxide and oxygen, but biomass balance criterion was focused on meeting the needs of the colonists in the habitat, especially for nitrogen. A second criterion involved assuring nutrition for the colonists in the Habitat.

Table 8. Nutrition by crop.

Crop	kg per kg live biomass per day Human Usable					
	Kcal	Carbohydrate	Fats	Proteins	Fiber	N
Rice	13.00	0.0025861	0.0000373	0.0004842	0.0002141	0.000068981
Tomato	13.14	0.0028058	0.0001443	0.0006239	0.00086553	0.000073532
Soybeans	15.89	0.0011382	0.0007004	0.0013339	0.0004326	0.000068959
Chlorophyta	3.77	0.0018400	0.0018400	0.0041400	0.0004600	0.0006099
Spirulina	14.99	0.0013894	0.0002239	0.0034015	0.000229654	0.00049435
Kelp	8.6	0.0018931	0.00011078	0.00029034	0.000257163	0.0000417932
Silver Carp	3.48	0.0000000	0.0001526	0.0004686	0.0000000	0.0000683
Tilapia	5.87	0.0000000	0.0001039	0.0012140	0.0000000	0.0001748
Shrimp	7.08	0.0000000	0.0000424	0.0016702	0.0000000	0.0002461

Primarily, the habitat needs biomass_out edible from the Hydroponic Stages (largely carbohydrate and fiber, some fats and protein), Aquatic Stages (largely Protein and Fat, carrying nitrogen), and oxygen from the Algae Reactor Stages, secondarily from the Hydroponic Stages. Hydroponic crops imply inedible biomass for other stages. Secondarily, the Hydroponic and Algae Reactor stages need carbon dioxide and biomass_excrete (ammonia) from the Habitat and Aquatic Stages, possibly from the Yeast-Bacteria Reactor stage, while the Aquatic Stages need oxygen from either the Algae Reactors (first) or Hydroponic Stages (second). Tertiary, Aquatic Stages need biomass_out (Proteins, fiber, fat, carbohydrate) from the Algae Stage, waste from the Hydroponic Stages, and lastly from the Yeast-Bacteria Stage.

Table 9. Human Habitat needs per person as fed to the simulator.

Habitat	kg per person per day Food Needs					
	Kcal	Carbohydrate	Fats	Proteins	Fiber	N
Habitat	2000	0.3240000	0.0703000	0.0500000	0.0250000	0.0073662

Further, then entire biomass is not consumed in each day. A resident biomass must exist to generate the harvest, and this is the living biomass tracked as population.

The non-crop biomass_out_growth is not human usable at all, but must be balanced in the farm, and the farm must be sized to accommodate it. Calculating the minimum crop population to meet the human needs in Table 7 above for each category, using the data in Table 6, we get the the next two tables:

Table 10. Minimum living mass population to meet each human nutrition category.

Crop	kg living mass to meet Habitat Needs for each person per category (no overgrowth)						Maximum Pop (living kg)
	Kcal	Carbohydrate	Fats	Proteins	Fiber	N	
Rice	153.81	125.29	1885.27	103.26	116.79	106.79	1885.27
Tomato	152.26	115.48	487.33	80.14	28.88	100.18	487.33
Soybeans	125.88	284.65	100.37	37.48	57.79	106.82	284.65
Chlorophyta	530.22	176.09	38.21	12.08	54.35	12.08	530.22
Spirulina	133.44	233.19	313.96	14.7	108.86	14.9	313.96
Kelp	232.57	171.15	634.6	172.21	97.21	176.25	634.6
Silver Carp	574.8	N/A	460.82	106.71	N/A	107.86	574.8
Tilapia	340.91	N/A	676.68	41.19	N/A	42.14	676.68
Shrimp	282.35	N/A	1658.91	29.94	N/A	29.94	1658.91

The maximum population for all species is driven by caloric need. Tomato, *Spirulina*, rice, soybeans, are all dense in food energy per growing kg per day, with *Chlorophyta* the best source of nitrogen and proteins among those (i.e. lowest biomass to achieve Nitrogen and Protein goals), soybeans and *Chlorophyta* the best among those for fats, tomatoes for carbohydrates. This seems counter intuitive, but due to the tomato plants (the size of small trees) being able to quickly fix carbons in fruit mass in lieu of vine or root, they produce carbohydrate quickly in fruit, versus soybeans and rice, which accumulate mass in stems and especially nodules in roots. If calories are ignored, *Spirulina* and *Chlorophyta* are top nitrogen/protein performers, which is why they were long suggested as a solution to hunger on Earth²⁹. These are not the only criteria for the farm of course. Oxygen, carbon dioxide and water are needed for the habitat, and the farm organisms, though this may not be a driver as shown in Table 11.

Table 11. Minimum Population (in living kg) to meet habitat inputs and outputs.

kg living mass to meet Habitat Needs for each person per day per category (no overgrowth)		
Crop	Habitat O2 in	Habitat CO2 out
Rice	65.007	66.816
Tomato	30.678	34.549
Soybeans	54.104	44.066
Chlorophyta	41.863	52.127
Spirulina	133.114	93.004
Kelp	198.275	203.866

A single crop is not a panacea either when it comes to meals. In order to meet caloric needs from any one of the crops examined, large wet masses would have to be consumed, beyond the inputs of normal humans, as shown in Table 12.

Table 12. Kilograms of Raw Wet Mass for 2,000 kcal.

Crop	kg of food (raw wet mass) to meet dietary kcal	kg of food (raw wet mass) to meet Nitrogen needs
Rice	0.56	0.39
Tomato	11.11	7.31
Soybeans	1.03	0.87
Chlorophyta	53.66	1.22
Spirulina	7.69	0.86
Kelp	4.65	3.52
Silver Carp	1.57	0.30
Tilapia	2.08	0.26
Shrimp	2.35	0.25

In addition to the culinary limitations of a single crop, colonists will want access to a diversity of foods for nutrition and emotional well being, so solutions were selected that favored a use of all nine crop species.

The balance and mass flows create a complex set of possibilities. Too much of the wrong crop might short-change oxygen or water, or allow too much carbon dioxide. Given this rule set, a series of random walks, in a Monte Carlo analysis, within the maximum ranges in the tables above, seeking a goal of balance, were run in a simulation. First, a broad range of populations was selected from zero to the maximum populations in Table 8, then those population sets approaching mass balance and meeting the nutrition requirements of the habitat were selected, and a range varied around those population sets. The range was tightened as balance was approached, and nitrogen tracked throughout. After 130,000 runs, a near solution was selected. Scoring of each run focused on human needs, then mass balance. The string of populations is the 'genome' of the possible solution, the balance and habitat scores, the trial. While only single solution was sought here, there were many possibilities, and the simulator, constructed in PERL and XML, can support a near unlimited number of species. The time to run the simulator for all runs was negligible.

III. Results

In nearly every run of the simulation, oxygen was in excess, due to the algae crops, corrected by the Yeast-Bacteria reactor in converting fiber to usable excreted and carbon dioxide. It was not difficult to meet the minimum human nutrition needs given many mixes of these species, other than calories, which drove higher populations. The best mass balancing solution found in this set of runs had elements of all nine crop species, though the result favored soybeans and shrimp as the runs progressed. The soybeans were favored likely due to the high nutrition content per edible biomass produced each day, and the the same can be said for the shrimp, though the simulator balanced far more complex variables then just food efficiency as noted in the sections above, to arrive at this solution set. A different set of runs with different population seeds may have resulted in a different population set.

Table 13. Near balance solution per person with diet and Yeast-Bacteria Reactor sizing.

Crop	Live kg of total organism	kg of food to Habitat per person (raw wet mass)	% kcal	Nitrogen in Food (kg)
Rice	2	0.01	1.30%	0.0001380
Tomato	2	0.15	1.31%	0.0001471
Soybeans	193	0.39	38.19%	0.0033149
Chlorophyta	5	0.51	0.94%	0.0030496
Spirulina	5	0.29	3.75%	0.0024718
Kelp	2	0.04	0.86%	0.0000836
Silver Carp	71	0.19	12.35%	0.0048490
Tilapia	25	0.15	7.33%	0.0043698
Shrimp	385	0.8	33.96%	0.0235946
Yeast-Bacteria Reactor	24.3			

Excess edible biomass from all photosynthetic crops but rice, kelp, and tomatoes had to be shunted to the animals in the system, in addition to inedible biomass that was processed and fed to the animal crops. The Yeast-Bacteria Reactor processed ~16 kg of mass, resulting in ~24 kg living biomass size. This is just one of many possible solution sets, though this solution allows for a fairly diverse menu using the approximately 2.5 kg of wet food mass per person per day, requiring around 715 kg of living crop organisms and Yeast-Bacteria biomass.

The simulator shows an inefficiency of roughly 0.004 kg/day for this solution due to lack of perfect mass balance. If this is resupply, for 100 colonists, this is roughly 140 kg/yr.

For initial supply for the farm sized for 100 colonists, an estimate made by multiplying the dry mass inputs for the living mass in Table 13 for one year, plus a day's living mass, plus seed (using best guess assumptions), yields Table 14.

Table 14. Initial supply estimate (assuming some in situ, with guesses).

Crop	living kg	Dry or Seed kg	Dry biomass inputs for 1 yr for this living mass (kg)	Total 1 year initial supply (kg)
Rice	200	87	5	292
Tomato	200	2	31	233
Soybeans	19300	193	4,697	24,190
Chlorophyta	500	5	88	593
Spirulina	500	5	38	543
Kelp	200	2	27	229
Silver Carp	7100	71	3,168	10,339
Tilapia	2500	25	25,000	27,525
Shrimp	38500	39	57,971	96,510
Yeast-Bacteria Reactor	2429.69	24	100	2,554
<i>Subtotal</i>				163,008
Add Structure	<i>(Assume 0.3 multiplier)</i>			48,902
TOTAL INITIAL MASS (kg)				211,910

A sizing estimate was made using a series of guesses for volumes for living mass from Table 13 above (except for the fish, where Ref. 34 was used to estimate volume, 50 kg/m³), resulting in Table 15.

Table 15: Estimated volumes and footprint for the farm to support 100 colonists

Crop	living kg	space ratio in living kg/m ³	m ³ for this living kg	multiplier for structure (total size of stage per living m ³)	stage volume in m ³	height (m)	Footprint (m ²)	Footprint (hectares)	Footprint (acres)
Rice	200	0.9	222.22	3	666.67	3	222.22	0.02	0.05
Tomato	200	0.5	400	3	1200	3	400	0.04	0.1
Soybeans	19300	0.5	38600	3	115800	3	38600	3.86	9.54
Chlorophyta	500	500	1	2	2	3	0.67	0.00	0.000165
Spirulina	500	500	1	2	2	3	0.67	0.00	0.000165
Kelp	200	10	20	2	40	3	13.33	0.00	0.003295
Silver Carp	7100	50	142	2.5	355	2	118.33	0.01	0.029241
Tilapia	2500	50	50	2.5	125	2	41.67	0.00	0.010296
Shrimp	38500	50	770	2.5	1925	2	641.67	0.06	0.158559
Yeast-Bacteria Reactor	2429.69	500	4.86	2	9.72	3	3.24	0.00	0.000801
Subtotal					120,125		40,042	4	10
Multiplier for between stages							1.5		
TOTAL SIZE					180,188		60,063	6.01	14.84

The farm size is driven by the soybeans in this solution, which require almost four hectares in a flat placement (though stacking of hydroponic beds could vastly lower the footprint). Total farm footprint is estimated here to be six hectares (roughly 15 acres), or a volume of 180,188 m³. This solution has less initial supply mass than in the prior paper¹, but nearly six times larger in footprint.

IV. Conclusion

There is still much work before the theoretical calculations in this paper, and the last 50 years of papers by many authors, become reality. That said, this paper provides a glimpse of the possibilities a diverse farm can provide, using the four stage types, i.e. Hydroponic, Aquatic, Algae Reactor, and Yeast-Bacteria Reactor. This set of species is a diverse possibility of menus. Obviously scale is a factor, given the crops selected, and large quantities will need to be grown to achieve the daily harvest assumption, with inefficiencies not considered here. Next steps for our analysis will be to increase fidelity by tracking all elements and minerals, and to begin collecting data for the reactor designs, including scale models of reactors, and development of control system components and rule sets.

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References

- ¹Meyer, B.L., "Of Fruits and Fishes: A Space Farm and Recycling Concept", AIAA SPACE 2015 Conference and Exposition, SPACE Conferences and Exposition, (AIAA 2015-4607)
- ²Wieser, M., Holden, N., Coplen, T., Böhlke, J., Berglund, M., Brand, W. et al. Atomic weights of the elements 2011 (IUPAC Technical Report), Pure Appl. Chem. Research Triangle Park, NC: International Union of Pure and Applied Chemistry, 2013.
- ³Jones, H. "Design Rules for Life Support Systems, 33rd International Conference on Environmental Systems (ICES)." ICES40, 2003-01 -2356. Vancouver, BC: Society of Automotive Engineers, Inc, 2003. <http://ntrs.nasa.gov/archive/nasa/casi.ntrs.nasa.gov/20040012725.pdf>.
- ⁴Guidance for Industry: A Food Labeling Guide, US Food and Drug Administration, January 2013 <http://www.fda.gov/downloads/Food/GuidanceRegulation/UCM265446.pdf>.
- ⁵Hausman, R. E.; Cooper, G. M. The cell: a molecular approach. 2004. Washington, D.C: ASM Press. ISBN 0-87893-214-3.
- ⁶Reeds, P.J., Dispensable and Indispensable Amino Acids for Humans, J. Nutr. July 1, 2000 vol. 130 no. 7 1835S-1840S.

⁷United States Department of Agriculture, Agricultural Research Service, National Nutrient Database for Standard Reference Release 28, May, 2016 Software v.2.6.1, <https://ndb.nal.usda.gov/ndb/foods>.

⁸Pearce, G.R., Lee, J.A., Simpson, R.J., Doyle, P.T., "Sources of variation in the nutritive value of wheat and rice straws", Plant breeding and the nutritive value of crop residues, JUNE 1988 INTERNATIONAL LIVESTOCK CENTRE FOR AFRICA, <http://www.fao.org/Wairdocs/ILRI/x5495E/x5495e00.htm>.

⁹Tang, L., Zhu, Y., Hannaway, D., Meng, Y., Liu, L., Chen, L., Cao, W., "RiceGrow: A rice growth and productivity model", NJAS - Wageningen Journal of Life Sciences, Volume 57, Issue 1, December 2009, Pages 83–92, doi:10.1016/j.njas.2009.12.003, <http://www.sciencedirect.com/science/article/pii/S1573521409000153>

¹⁰ed. Manns, R., Schmidt, S., Beveridge, C., PLANTS IN ACTION A resource for teachers and students of plant science, 2nd ed., © Australian Society of Plant Scientists, New Zealand Society of Plant Biologists, and New Zealand Institute of Agricultural and Horticultural Science 2010–2016, <http://plantsinaction.science.uq.edu.au/content/about>

¹¹Xing, Y. Wang, R., Sun, W., An, J., Wang, C.X., H.J. Bao, L. Gong, X. Z. Wang, "Effect of Balanced Fertilization on Rice Nutrient Uptake, Yield, and Profit", Better Crops/Vol. 93 (2009, No. 1), [http://www.ipni.net/publication/bettercrops.nsf/0/5C6E09CE82D30C73852579800070212E/\\$FILE/Better%20Crops%202009-1%20p4.pdf](http://www.ipni.net/publication/bettercrops.nsf/0/5C6E09CE82D30C73852579800070212E/$FILE/Better%20Crops%202009-1%20p4.pdf)

¹²Wu, C., Feng, Y., Shohag, M. J. I., Lu, L., Wei, Y., Gao, C., and Yang, X., "Characterization of 68Zn uptake, translocation, and accumulation into developing grains and young leaves of high Zn-density rice genotype", J Zhejiang Univ Sci B. 2011 May; 12(5): 408–418. doi: 10.1631/jzus.B1000291, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3087098/>.

¹³Mueller, N., "Plant Nutrient Analysis: Do your soybeans have the right stuff?" 7/25/2013, iGrow, South Dakota State University Brookings, SD. <http://igrow.org/agronomy/profit-tips/plant-nutrient-analysis-do-your-soybeans-have-the-right-stuff/#sthash.eHjNCXoD.dpuf>.

¹⁴"Soybean Growth and Development" February 19, 2015, Corn Agronomy, University of Wisconsin, 1575 Linden Drive - Agronomy, Madison WI 53706, <http://corn.agronomy.wisc.edu/Crops/Soybean/Default.aspx>

¹⁵Pedersen, P., "Soybean Growth Stages", ISU Extension publication, Soybean Growth and Development, 4/9/07, Copyright 2003-2016, Iowa State University Extension.

¹⁶Hill, W., Pegg, M. Evaluating Asian Carp Colonization Potential and Impact in the Great Lakes Final Report to Illinois-Indiana Sea Grant. Champaign, IL: Sea Grant, 2008.

¹⁷Kapetsky, J.M.; Nath, S.S. A strategic assessment of the potential for freshwater fish farming in Latin America. COPESCAL Technical Paper. No. 10. Rome, FAO. 1997. 128p, ISBN 92-5-103989-5 <http://www.fao.org/docrep/005/W5268E/W5268E00.htm#TOC>

¹⁸Rakocy, J.E., Tank Culture Of Tilapia, The Fish Site, 5m Publishing, 01 Nov 2005, Sheffield, S35 1QN, England. <http://www.thefishsite.com/articles/136/tank-culture-of-tilapia/>

¹⁹Kungvankij, P., and Chua, T.E., Shrimp Culture: Pond Design, Operation And Management, NACA Training Manual Series No. 2, Food and Agriculture Organization of the United Nations and Network of Aquaculture Centres in Asia (NACA), June 1986, <http://www.fao.org/docrep/field/003/ac210e/AC210E00.htm#TOC>.

²⁰Walker, S. J., ECOPHYSIOLOGY OF GROWTH IN THE PACIFIC WHITE SHRIMP (LITOPENAEUS VANNAMEI), Dissertation, Texas A&M University, May 2009

²¹Davis, D. A., Rouse, D., Amaya, E., Zelaya, O., Venero, J., Quintero, H., "Historical Review Of Feeding Protocols For The Pacific White Shrimp Litopenaeus Vannamei At Claude Peteet Mariculture Center, Gulf Shores, Alabama", AQUA 2006, World Aquaculture Society, Louisiana State University, Baton Rouge, LA, 2006.

²²Reed D.C., Rassweiler A, Arkema K.K., "Biomass rather than growth rate determines variation in net primary production by giant kelp", Ecology. 2008 Sep; 89(9):2493-505.

²³Danesi, E.D., Rangel-Yagui, C.O., Sato, S., Monteiro de Carvalho, J.C., "Growth and Content of Spirulina Platensis Biomass Chlorophyll Cultivated at Different Values of Light Intensity and Temperature Using Different Nitrogen Sources", Braz J Microbiol. 2011 Jan-Mar; 42(1): 362–373. doi: 10.1590/S1517-83822011000100046, <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3768952/>.

²⁴Pena, J. "Greenhouse Vegetable Production Economic Considerations, Marketing, and Financing." 30 05 2005. Texas A&M AgriLife Extension Service. 2015. <http://aggie-horticulture.tamu.edu/greenhouse/hydroponics/economics.html>.

²⁵Productivity: Crop Water Information: Tomato, WATER, FAO, 2015 http://www.fao.org/nr/water/cropinfo_tomato.html

²⁶Ulissi, V., Antonucci, F., Benincasa P., Farneselli M., Tosti G., Guiducci M., Tei F., Costa C., Pallottino F., Pari L. and Menesatti P., "Article Nitrogen Concentration Estimation in Tomato Leaves by VIS-NIR Non-Destructive Spectroscopy", Sensors 2011, 11, 6411–6424; doi:10.3390/s110606411 sensors ISSN 1424-8220 www.mdpi.com/journal/sensors

²⁷Van Hoek, P., Van Dijken, J.P., and Pronk, J.T. "Effect of Specific Growth Rate on Fermentative Capacity of Baker's Yeast", Appl Environ Microbiol. 1998 Nov; 64(11): 4226–4233.

²⁸Doucha, J., Straka, F., Livansky, K. "Utilization of flue gas for cultivation of microalgae (Chlorella sp.) in an outdoor open thin-layer photobioreactor." Journal of Applied Phycology (2005): 403–412.

²⁹Phukana M.M., Chutiab R.S., Konwara, B.K., Katakai, R. "Microalgae Chlorella as a potential bio-energy feedstock", Applied Energy 88(10):3307-3312 · September 2011

³⁰Belasco, Warren (July 1997). "Algae Burgers for a Hungry World? The Rise and Fall of Chlorella Cuisine". Technology and Culture 38(3): 608–34. doi:10.2307/3106856. JSTOR 3106856, <https://www.jstor.org/stable/3106856>.

³¹"Seaweed, Chlorella, Dried" Nutrition facts, SELF Magazine, 2014 Condé Nast. <http://nutritiondata.self.com/facts/custom/569428/2>.

³²Csavina JL, Stuart BJ, Riefler RG, Vis ML., "Growth optimization of algae for biodiesel production", *J Appl Microbiol.* 2011 Aug;111(2):312-8. doi: 10.1111/j.1365-2672.2011.05064.x. Epub 2011 Jun 23.

³³Lürling, M., Eshetu, F., Faassen, E. J., Kosten, S. And Huszar, V. L. M. (2013), Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology*, 58: 552–559. doi:10.1111/j.1365-2427.2012.02866.x.

³⁴Seltz, J. MEDITERRANEAN REGIONAL AQUACULTURE PROJECT, TECHNICS USED FOR INTENSIVE REARING AND ALIMENTATION OF FISH AND SHELLFISH, "Rearing Structures For Aquaculture" Ch. 10. Motta di Livenza, Italy: Food and Argicultural Organization of the United Nations, 1986.