Supplementary Information

S1. Supplementary Discussion

UV-C Irradiation Experiments

The specific nature and mechanism of the urea-forming reaction is being a subject of debate [1–4]. It is widely accepted that the synthesis of cyanate (OCN^-) plays a crucial role in the formation of urea[5]. Experimental studies have suggested that OCN^- could be formed by photooxidation of CN^- ions and hydrolysis of cyanogen $(CN)_2$ (e.g., [6,7]). However, it is important to note that the rate of CN^- photooxidation under UV irradiation alone is significantly slow[6], and therefore the contribution of CN^- to OCN^- formation may be minimal. Furthermore, as shown in this study, increasing the concentration of CN^- decreases the urea yield, confirming that CN^- photooxidation is not the main factor in urea formation.

This study demonstrated the importance of ferrocyanides in the urea formation process. CN radicals (CN^*) required for the formation of $(CN)_2$ (reaction 4) can be generated from the degradation of pentacyanoaquaferrate(II) $[Fe(CN)_5H_2O]^{3-}$ complex[7]. However, ferrocyanide $[Fe(CN)_6]^{4-}$ can only generate $[Fe(CN)_5H_2O]^{3-}$ by photoaquation at long wavelengths (>300 nm) in the UV spectrum [8]. Therefore, under the given 254 nm irradiation conditions examined in this study, the preferred reaction is the photooxidation of $[Fe(CN)_6]^{4-}$ to ferricyanide $[Fe(CN)_6]^{3-}$ [9] (reaction 1) rather than the formation of $[Fe(CN)_5H_2O]^{3-}$. The resulting $[Fe(CN)_6]^{3-}$ can be gradually degraded by reaction with OH^* (reaction 2) to produce CN^* (see equations 21-26 in [10]). The reddish-brown precipitate observed at the end of the UV-exposure experiment could be the iron oxyhydroxide complexes $[Fe(OH)_x(H_2O)_y]$ resulting from the reaction between the final degraded product, the hexaaquairon complex $[Fe(H_2O)_3]^{3+}$ with hydroxyl (OH^-) ions (reaction 3).

$$[Fe(CN)_6]^{4-} \xrightarrow{h\mu} [Fe(CN)_6]^{3-} + e^- \text{ -------------------------------}(1)$$

$$[Fe(CN)_6]^{3-} + HO^* \rightarrow [Fe(CN)_5]^{2-} + HO^- + CN^* \text{-------------}(2)$$

$$[Fe(H_2O)_3]^{3+} + xOH^- \rightarrow [Fe(OH)_xH_2O_{3-x}]^{(3+)-x} \text{--------------}(3)$$

$$CN^* + CN^* \rightarrow (CN)_2 \text{---}(4)$$

$$(CN)_2 + 2OH^- \rightarrow CN^- + OCN^- + H_2O \text{----------------------------}(5)$$

$$OCN^- + 2H_2O \rightarrow CO_3^{2-} + NH_4^+ \text{--------------------------------------}(6)$$

$OCN^{-} + NH_4^{+} \rightarrow H_2NCONH_2$---------------------------------------(7)

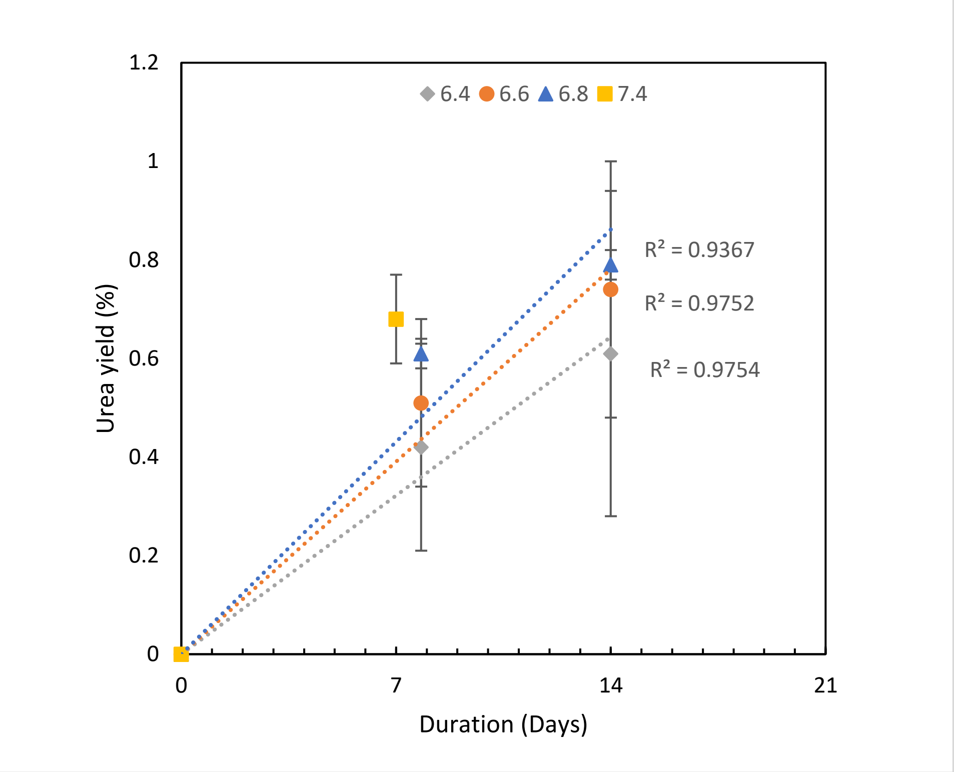
The constant rate of urea production observed (over 28 days) despite a low ferrocyanide concentration (0.06 mmol L^{-1}) (Figure 1a) indicates that either the degradation of $[Fe(CN)_6]^{3-}$ is slow or iron acts as a catalyst, with $[Fe(CN)_6]^{3-}$ being regenerated with excess CN^- present in the media. If iron is indeed acting as a catalyst, the reaction can be expected to continue until all cyanides are converted to urea.

The observed inverse relationship between CN^- concentration and urea yields (Figure 1b and 1d) confirms that the contribution of CN^- photooxidation to the formation of OCN^- was minimal. This inverse relationship could be due to the stabilization of $(CN)_2$, which prevents the formation of OCN^-. Once OCN^- is formed, urea, carbamate, and carbonate can be formed by parallel reactions (reactions 6 and 7;[4]).

The presence of NH_4^+ in the starting solution can increase the rate of urea synthesis [4]. The NH_4^+ also can be generated by the hydrolysis of OCN^- itself via a parallel reaction (reaction 6). Therefore, the presence of NH_4^+ in the starting medium may limit OCN^- dissociation into CO_3^{2-} (reaction 6) and thus increase the fraction of OCN^- available for urea production. The reason why high urea yields were obtained in this study when the NH_4^+ concentration of the starting solution was high could be due to these mechanisms. Though, a linear correlation was observed with NH_4^+ concentration and urea yields at high CN^- concentration (1.36 mmol L^{-1}), it was not linearly correlated at low CN^- concentration (0.46 mmol L^{-1}) (Figure 1b).

In addition, the pH of the medium affects urea formation. Slightly alkaline solutions enhance the rate and stability of ferrocyanide formation[11], resulting in a higher urea yield compared to slightly acidic mediums. It is important to note that since pH buffering was not performed during the experiments, the pH may have increased over time due to urea formation. At the proposed Archean pH values (6.4-7.8[12]) the HCN/CN^- equilibrium could have shifted more towards HCN (pKa HCN = 9.3). As envisaged by our experiments lowering the CN^- concentration should positively affect for urea production (Figure 1d). But the observations suggest that lowering pH from 8.4 to 7.4 and 6.4 results 16% and 60% decrease respectively in urea yields (see experiments C, P and S in Supplementary Table 1) for 7-day exposure. Since ferrocyanide complexes are stable at this pH range[11] the factor that controls urea yield should be governed by the reaction 5. Low stability of the cyanate ion can explain these results as when the pH is low OCN^- will convert into NH_4^+ ($OCN^- + 2H^+ + 2H_2O \rightarrow NH_4^+ + H_2CO_3$, [13]). However once urea is formed it is stable even at acidic pH values[14] and therefore it can accumulate at slower rate compared to high pH. However, in more alkaline (pH ≥ 9) urea formation rate could be slow due to low rates of ferrocyanide formation[11] and the degradation of urea into NH_4^+ and OCN^-[13].

In summary, the photosynthetic formation of urea is favoured under alkaline pH conditions with the availability of NH_4^+ and ferrocyanide. A similar experiment using concentrated solutions of NaCN (0.2 mol L^{-1}) and NH_4^+ (0.5 mol L^{-1}) obtained higher yields[7], but a direct comparison is not feasible since the previous study determined the urea yields through a visual comparison of paper chromatograms. In addition, the occurrence of such concentrated solutions in the Archean Ocean is unlikely considering their production rates.



Supplementary Figure 1 – Formation yields of urea (as percentage of initial CN^- concentration) at pH 6.4 -7.4.

Supplementary Table 1 - Experimental conditions for the UV irradiation experiments and the urea yields

Experiment	NaCN (mmol L^{-1})	NH_4Cl (mmol L^{-1})	$K_4Fe(CN)_6$ (mmol L^{-1})	Initial pH	Duration (Days)	Urea (µmol L^{-1})	2SD	% CN converted to urea	2SD	N
A	1	0.1	0.06	8.4	7	2.11	0.25	0.16	0.02	3
					14	4.27	0.09	0.31	0.01	3
B	1	0.5	0.06	8.4	7	6.26	0.28	0.46	0.02	3
C	1	1	0.06	8.4	7	11.04	0.77	0.81	0.06	3
					14	24.22	2.94	1.78	0.22	3
					21	39.15	4.59	2.88	0.34	3
					28	64.11	9.11	4.49	0.67	3
D	1	3	0.06	8.4	7	34.99	2.30	2.57	0.17	3
E	1	5	0.06	8.4	7	49.64	2.86	3.65	0.21	3
					14	83.50	4.21	6.14	0.31	3
F	0.1	3	0.06	8.4	7	22.92	3.25	4.98	0.71	2
G	0.1	5	0.06	8.4	7	21.44	1.31	4.66	0.29	3
H	0.1	1	0.06	8.4	7	10.72	0.25	2.33	0.05	3
I	0.1	1	0.06	8.4	7	8.77	3.61	1.91	0.79	3
J	3	1	0.06	8.4	7	8.61	1.12	0.26	0.03	3
K	5	1	0.06	8.4	7	18.60	4.97	0.35	0.09	3
L	8	1	0.06	8.4	7	22.54	6.30	0.27	0.08	3
M	1	1	0.12	8.4	7	19.58	3.61	1.14	0.21	3
N	1	1	0	8.4	7	2.63	0.38	0.26	0.04	3
O	1	1	0.06	6.4	7	Below detection limit				3
P	1	1	0.06	7.4	7	9.27	1.25	0.68	0.09	3
Q	1	1	0.06	6.4	8	5.65	2.90	0.42	0.21	3
					14	8.35	4.52	0.61	0.33	3
R	1	1	0.06	6.6	8	6.92	2.32	0.51	0.17	3
					14	10.03	3.48	0.74	0.26	3
S	1	1	0.06	6.8	8	8.23	0.46	0.61	0.03	3
					14	10.77	0.46	0.79	0.03	3

S2. Timing for accumulation of urea in Archean Oceans

In the natural system, the UV-C penetration depth of a water column is determined by the concentration of dissolved and particulate matter and the water itself[15,16]. The available limited data suggest that UV-C (254 nm) penetrates >> 80 meters before it gets fully absorbed in water columns having Fe^{3+} concentration similar to Archean conditions (see Fig 6 of [17]). Studies also reveal that the attenuation of UV wavelengths >240 nm mostly occurs due to I^- and $[Fe(CN)_6]^{4-}$ in the open ocean conditions[15]. Still, these two components are capable of aiding the urea formation[7]. Thus, these regions become high-productivity regions for urea-like photosynthetic molecules. Additionally, the ocean mixing times reported for Archean (1.3 kyrs [18]) would allow the accumulation of high ΣHCN concentration in the topmost waters. This will eventually produce higher urea yield in contrast to what was predicted in this study where homogenization of deposited HCN in the whole ocean (covering the entire Earth) was assumed. Thus, cyanobacteria, which are an epipelagic organism (species living near the surface of the ocean) would have been severely affected and the time to accumulate harmful levels of urea in these cyanobacterial habitable areas could have been faster than anticipated in this work even if its production is confined to the top layers of the ocean. However, a scenario with faster ocean mixing with low penetration of UV-C irradiation in the water column will lengthen the time needed to build up urea concentrations (Supplementary Table 3). However, it is still possible to produce harmful concentrations of urea within short time scales under conditions where UV-C penetration occurs only up to 30 m depth (Supplementary Table 3).

Supplementary Table 2 – Time taken to build 2 mmol L^{-1} of urea at various pH, temperatures and HCN deposition rates under homogenous urea production in the Archean ocean.

pH = 8.4				
Temperature (°C)	HCN deposition rate (nmol cm^{-2} yr^{-1})	ΣHCN (nmol L^{-1})	Time taken to build 2 mmol L^{-1} urea (× 10^{6} years)	
			% of cyanides converted into urea	
			0.05%	2.50%
23	50	5.62	15	0.3
	100	11.24	7	0.15
	200	22.49	4	0.07
	300	33.73	2	0.05
	400	44.98	2	0.04
	500	56.22	1	0.03
50	50	0.07	>1000	25
	100	0.13	626	13
	200	0.27	313	6
	300	0.40	209	4
	400	0.53	157	3
	500	0.67	125	3
75	50	<0.01	>1000	593
	100	0.01	>1000	297
	200	0.01	>1000	148
	300	0.02	>1000	99
	400	0.02	>1000	74
	500	0.03	>1000	59

pH = 7.4				
Temperature (°C)	HCN deposition rate (nmol cm^{-2} yr^{-1})	ΣHCN (nmol L^{-1})	Time taken to build 2 mmol L^{-1} urea (× 10^{6} years)	
			% of cyanides converted into urea	
			0.05%	2.50%
23	50	50.29	1.66	0.03
	100	100.59	0.83	0.02
	200	201.18	0.41	0.01
	300	301.77	0.28	0.01
	400	402.36	0.21	<0.01
	500	502.94	0.17	<0.01
50	50	0.46	180.66	3.61
	100	0.92	90.33	1.81
	200	1.85	45.17	0.90
	300	2.77	30.11	0.60
	400	3.69	22.58	0.45
	500	4.61	18.07	0.36
75	50	0.01	>1000	120.46
	100	0.03	>1000	60.23
	200	0.06	>1000	30.12

	300	0.08	>1000	20.08
	400	0.11	752.90	15.06
	500	0.14	602.32	12.05

pH = 6.4				
Temperature (°C)	HCN deposition rate (nmol cm^{-2} yr^{-1})	ΣHCN (nmol L^{-1})	Time taken to build 2 mmol L^{-1} urea (× 10^{6} years)	
			% of cyanides converted into urea	
			0.05%	2.50%
23	50	497.02	0.17	<0.01
	100	994.04	<0.1	<0.01
	200	1988.08	<0.1	<0.01
	300	2982.12	<0.1	<0.01
	400	3976.16	<0.1	<0.01
	500	4970.20	<0.1	<0.01
50	50	4.41	18.90	0.38
	100	8.82	9.45	0.19
	200	17.64	4.73	<0.1
	300	26.45	3.15	<0.1
	400	35.27	2.36	<0.1
	500	44.09	1.89	<0.1
75	50	0.12	671.5	13.43
	100	0.25	335.75	6.71
	200	0.50	167.87	3.36
	300	0.74	111.92	2.24
	400	0.99	83.94	1.68
	500	1.24	67.15	1.34

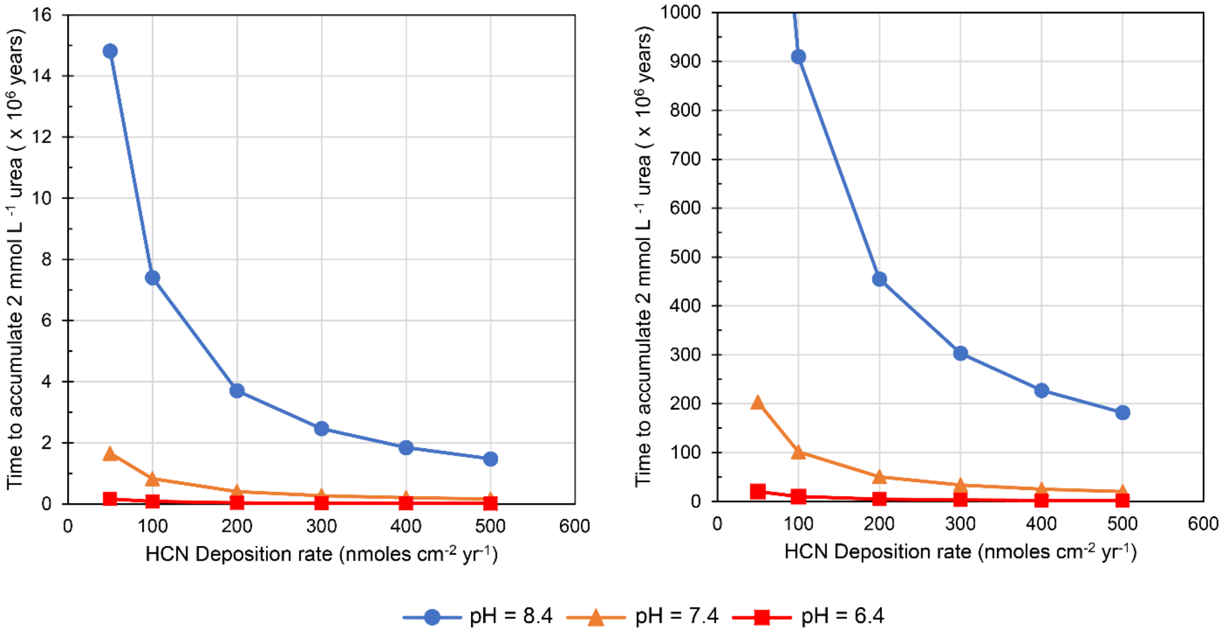
Supplementary Table 3 - Time taken to build 2 mmol L^{-1} of urea at various pH, temperatures and HCN deposition rates considering a UV-C penetration depth of 30 m (0.81 % of average oceanic depth) in the Archean ocean.

pH = 8.4				
Temperature (°C)	HCN deposition rate (nmol cm^{-2} yr^{-1})	ΣHCN (nmol L^{-1})	Time taken to build 2 mmol L^{-1} urea (× 10^6 years)	
			% of cyanides converted into urea	
			0.05%	2.50%
23	50	5.62	>1000	36.38
	100	11.24	909.62	18.19
	200	22.49	454.81	9.10
	300	33.73	303.21	6.06
	400	44.98	227.41	4.55
	500	56.22	181.92	3.64
50	50	0.07	>1000	>1000
	100	0.13	>1000	>1000
	200	0.27	>1000	768.80
	300	0.40	>1000	512.53
	400	0.53	>1000	384.40
	500	0.67	>1000	307.52
75	50	<0.01	>1000	>1000
	100	0.01	>1000	>1000
	200	0.01	>1000	>1000
	300	0.02	>1000	>1000
	400	0.02	>1000	>1000
	500	0.03	>1000	>1000

pH = 7.4				
Temperature (°C)	HCN deposition rate (nmol cm^{-2} yr^{-1})	ΣHCN (nmol L^{-1})	Time taken to build 2 mmol L^{-1} urea (× 10^6 years)	
			% of cyanides converted into urea	
			0.05%	2.50%
23	50	50.29	203.36	4.07
	100	100.59	101.68	2.03
	200	201.18	50.84	1.02
	300	301.77	33.89	0.68
	400	402.36	25.42	0.51
	500	502.94	20.34	0.41
50	50	0.46	>1000	443.46
	100	0.92	>1000	221.73
	200	1.85	>1000	110.87
	300	2.77	>1000	73.91
	400	3.69	>1000	55.43
	500	4.61	>1000	44.35
75	50	0.01	>1000	>1000
	100	0.03	>1000	>1000
	200	0.06	>1000	>1000

	300	0.08	>1000	>1000
	400	0.11	>1000	>1000
	500	0.14	>1000	>1000

pH = 6.4				
Temperature (°C)	HCN deposition rate (nmol cm^{-2} yr^{-1})	ΣHCN (nmol L^{-1})	Time taken to build 2 mmol L^{-1} urea (× 10^{6} years)	
			% of cyanides converted into urea	
			0.05%	2.50%
23	50	497.02	20.58	0.41
	100	994.04	10.29	0.21
	200	1988.08	5.14	0.10
	300	2982.12	3.43	< 0.1
	400	3976.16	2.57	< 0.1
	500	4970.20	2.06	< 0.1
50	50	4.41	>1000	46.40
	100	8.82	>1000	23.20
	200	17.64	579.96	11.60
	300	26.45	386.64	7.73
	400	35.27	289.98	5.80
	500	44.09	231.99	4.64
75	50	0.12	>1000	>1000
	100	0.25	>1000	824.15
	200	0.50	>1000	412.08
	300	0.74	>1000	274.72
	400	0.99	>1000	206.04
	500	1.24	>1000	164.83



Supplementary Figure 2 – HCN deposition rate-time plots for the accumulation of 2 mmol L^{-1} urea at different pH at constant temperature of 23 °C and at a urea yield of 0.05%. (a) Considering homogenous urea production at all depths in the ocean (left) (b) Considering urea formation only at the top 30 m of the ocean (right).

S3. The composition of the bacterial growth medium

Supplementary Table 4 - Composition of A+ medium, note that original A+ medium was modified by omitting EDTA and NO_3^-.

Component (Assay)	Concentration in growth media (mmol L^{-1})
NaCl (99.5%)[1]	3.08×10^2
$MgSO_4.7H_2O$ (99.5%)[1]	2.00×10^1
$CaCl_2.2H_2O$ (99%)[1]	2.52×10^0
Triz Base (pH 8.2) (99%)[1]	8.26×10^0
$Na_2S_2O_3.5H_2O$ (99%)[1]	1.21×10^1
H_2NCONH_2 (98%)[2]	varied
KCl (99.99%)[3]	8.05×10^0
KH_2PO_4 (99.5%)[3]	3.70×10^{-1}
Trace Elements	
H_3BO_3 (99.5%)[1]	5.55×10^{-1}
$ZnCl_2$ (98%)[1]	2.3×10^{-3}
$FeCl_3.6H_2O$ (99%)[1]	1.4×10^{-2}
$MnCl_2.4H_2O$ (99%)[1]	2.2×10^{-2}
$CuSO_4.5H_2O$ (99.5%)[1]	1.2×10^{-5}
$CoCl_2.6H_2O$ (99%)[1]	5.0×10^{-5}
Vitamin B_{12} (>98%)[4]	3.0×10^{-6}
MoO_3 (99.5%)[3]	2.1×10^{-4}
$NiCl_2.6H_2O$ (98%)[3]	varied

Manufacture of reagents: [1] Fujifilm Wako Pure Chemical Corporation, Japan, [2] MP Biomedicals, France, [3] Kanto Chemical Co., Inc. Japan, *[4] Sigma Aldrich. Co, USA.*

S4. Experimental parameters used for bacterial cultures

Supplementary Table 5 - Growth Conditions used throughout the experiments. In sample names U, N, A stands for urea, Ni and agar respectively. The subscripts denote the concentration in mmol L^{-1}*unit for urea and nmol* L^{-1} *for nickel. The Ni blank in agar and liquid medium were 38 nmol* L^{-1} *and 36 nmol* L^{-1} *respectively.*

Experiment	Sample Name	Ni concentration (nmol L^{-1})[1]	Urea Concentration (mmol L^{-1})
A (Liquid)	U_2N_{36}	36	2
	U_2N_{86}	86	
	U_2N_{136}	136	
	U_2N_{236}	236	
B (Liquid)	U_2N_{236}	236	2
	U_4N_{236}		4
	U_6N_{236}		6
	U_8N_{236}		8
C (Agar)	$U_{0.001}N_{1038}A$	1038	0.001
	$U_1N_{1038}A$		1
	$U_5N_{1038}A$		5
	$U_{10}N_{1038}A$		10
	$U_{50}N_{1038}A$		50
	$U_{50}N_{88}A$	88	
	$U_{50}N_{48}A$	48	
	$U_5N_{48}A$		5

[1] After blank correction

S5. Bacterial Growth

Supplementary Table 6 - Optical Densities (750 nm) of bacterial cultures grown in different growth media.

Time after inoculation (Days)	U_2N_{36}	U_2N_{86}	U_2N_{136}	U_2N_{236}
0	0.011	0.016	0.012	0.011
1	0.015	0.028	0.018	0.013
2	0.031	0.068	0.041	0.026
3	0.052	0.121	0.105	0.048
4	0.084	0.212	0.185	0.080
5	0.146	0.313	0.250	0.136
6	0.236	0.384	0.320	0.210
7	0.332	0.415	0.358	0.275
8	0.360	0.424	0.399	0.332
9	0.362	0.432	0.444	0.501
10	0.379	0.427	0.445	0.514
11	0.371	0.441	0.448	0.521
12	0.360	0.448	0.442	0.503
13	0.353	0.445	0.434	0.480
14	0.341	0.443	0.433	0.451
15	0.328	0.442	0.433	0.442

Time after inoculation (Days)	U_2N_{236}	U_4N_{236}	U_6N_{236}	U_8N_{236}
0	0.007	0.006	0.007	0.006
1	0.008	0.010	0.008	0.006
2	0.014	0.014	0.012	0.012
3	0.021	0.022	0.021	0.018
4	0.034	0.030	0.028	0.029
5	0.061	0.046	0.041	0.043
6	0.108	0.074	0.066	0.074
7	0.153	0.116	0.100	0.104
8	0.212	0.179	0.149	0.129
9	0.269	0.251	0.183	0.131
10	0.256	0.300	0.197	0.111
11	0.267	0.338	0.190	0.099
12	0.259	0.361	0.177	0.090
13	0.285	0.350	0.174	0.083
14	0.311	0.334	0.164	0.079
15	0.296	0.314	0.157	0.073

Supplementary Table 7 Chlorophyll a Concentration Measurement

	A_{665}	A_{720}	Chl a (ug/ml)*	OD_{750} (sample)	Chl a (Normalized to OD)	Average	2sd (N=3)
MeOH	0.042	0.043					
15 days old culture							
U_2N_{236}	0.456	0.103	1.83	0.296	6.192	5.90	0.86
	0.36	0.052	1.60		5.405		
	0.408	0.06	1.81		6.105		
U_4N_{236}	0.337	0.074	1.37	0.314	4.353	4.18	0.30
	0.315	0.066	1.29		4.123		
	0.306	0.06	1.28		4.073		
U_6N_{236}	0.086	0.061	0.13	0.157	0.857	0.74	0.21
	0.068	0.048	0.11		0.693		
	0.071	0.052	0.10		0.660		
U_8N_{236}	0.058	0.058	0.01	0.073	0.071	0.17	0.22
	0.051	0.05	0.01		0.142		
	0.072	0.069	0.02		0.284		
18 days old culture							
U_2N_{236}	0.526	0.048	2.48	0.402	6.170	6.19	0.15
	0.532	0.046	2.52		6.273		
	0.521	0.046	2.46		6.131		
U_4N_{236}	0.070	0.047	0.12	0.206	0.603	0.54	0.13
	0.063	0.045	0.10		0.478		
	0.066	0.046	0.11		0.528		

*Dilution factor – 0.4
chlorophyll a (μg mL^{-1}) = 12.9447 × (A_{665} – A_{720}) (A_i = absorbance at i wavelength)

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