**Comparison of biocrude oil production from self-settling and non-settling microalgae biomass produced in the Qatari desert environment**

Table S1: Some details of the strains used in this study

|  |  |  |
| --- | --- | --- |
| Strain name | Magnified image of the strain | Calibration curve of optical density vs. biomass  X = optical density at 750 nm  Y = biomass density (g/L) |
| *Chlorocystis* sp. |  | Y= 0.7287x (r2 = 0.9721) |
| *Picochlorum* sp. | d:\Users\probir.das\Desktop\microscopic image\3C 100X b.jpg | Y= 0.629x-0.0305 (r2 = 0.9834) |

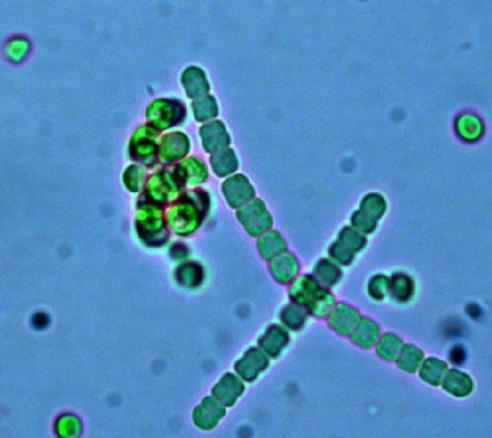
 

Figure S1: Contaminated culture of *Picochlorum* sp. culture (left); microscopic image of invading cyanobacteria (right).

Table S2: Nutrients added in 25,000 L raceway ponds for growing *Chlorocystis* sp. and *Picochlorum* sp. in natural seawater (42 ppt salinity):

|  |  |
| --- | --- |
| **Nutrients** | **Amount**  **(g)** |
| Urea (CON2H4) | 1500 |
| Sodium hydrogen phosphate (monobasic) NaH2PO4 | 150 |
| Ferric chloride FeCl3.6H2O | 2.5 |
| Zinc sulfate ZnSO4.7H2O | 0.55 |
| Sodium Molybdate NaMoO4.4H2O | 0.15 |
| Cobalt chloride CoCl2.6H2O | 0.25 |
| Copper sulfate CuSO4.5H20 | 0.25 |
| Manganese chloride MnCl2.4H2O | 0.45 |
| Thiamine.HCl | 2.5 |
| Biotin | 0.013 |
| Vitamin B12 | 0.013 |

Table S2a: Percent distribution of alkanes and other compounds in biocrude.

|  |  |  |  |
| --- | --- | --- | --- |
| *Chlorocystis* sp. Alkanes and others | Percent | *Picochlorum sp.* Alkanes and others | Percent |
| nonane | 9.66 | nonane | 15.77521 |
| decane | 13.33 | decane | 21.75798 |
| octane | 5.94 | octane | 9.70032 |
| cyclopropane | 0.166 | cyclopropane | 0.270959 |
| nonadecane | 0.149 | nonadecane | 0.243863 |
| Heptane | 4.48 | Heptane | 3.061833 |
| Undecane | 6.242736178 | Undecane | 8.426814 |
| Tridecane | 0.863357131 | Tridecane | 0.839972 |
| Hexadecane | 5.395982069 | Hexadecane | 1.354793 |
| pentadecane | 6.6412087 | pentadecane | 2.628299 |
| Cyclohexane | 23.67590902 | Cyclohexane | 15.25497 |
| Tridecane | 1.04599037 | Tridecane | 0.569013 |
| tetradecane | 0.66412087 | tetradecane | 0.541917 |
| Hexatriacontane | 0.879960153 | Hexatriacontane | 1.436081 |
| Cyclopentane | 10.37688859 | Cyclopentane | 5.744323 |
| 1,2,3-trimethylcyclohexane | 2.845757928 | 1,2,3-trimethylcyclohexane | 4.644231 |
| Dodecane | 1.859538436 | Dodecane | 2.655395 |
| Nonadecane | 0.348663457 | Nonadecane | 0.569013 |
| Docosane | 0.332060435 | Docosane | 0.541917 |
| Heptadecane | 3.03835298 | Heptadecane | 3.983092 |
| Tridecane | 0.215839283 |  |  |
| Tetrapentacontane | 0.913166196 |  |  |
| Heneicosan | 0.713929935 |  |  |
| teracosane | 0.205877 |  |  |

Table S2b: Percent distribution of alkenes, polyaromatics and other compounds in biocrude.

|  |  |  |  |
| --- | --- | --- | --- |
| alkenes, polyaromatics and others *Chlorocystis* | Percent | alkenes, polyaromatics and others *Picochlorum* | Percent |
| 2-Octanone | 0.300717 | Benzoic acid | 3.237268 |
| Thiazole | 1.896831 | Benzene | 49.19068 |
| 1-Pentanol | 0.323849 | 1-Octanol | 0.473747 |
| 2,5-Hexanediol | 0.393245 | 1-Octene | 22.50296 |
| Cyclohexanone | 4.3257 | Decanedioic acid | 0.789578 |
| Benzoic acid | 2.058755 | Ethanone | 3.395184 |
| Benzene | 17.07148 | Phenol | 13.89657 |
| 3-Eicosene | 0.902151 | 1-Undecene | 0.908014 |
| 1-Octene | 18.50567 | Decanedioic acid | 1.737071 |
| Tridecanol | 13.92551 | Phosphonic acid | 3.355705 |
| Ethanone | 2.66019 | METHYL 5-HYDROXY-6,6-DIMETHYL-3-OXOheptanoate | 0.513225 |
| 2-Undecene | 0.231321 |  |  |
| 3-Dodecene | 0.185057 |  |  |
| Phenol | 18.18182 |  |  |
| Decanedioic acid | 12.72265 |  |  |
| Heptene | 1.457321 |  |  |
| Hexadecanoic acid | 0.208189 |  |  |
| 7-Hexadecene | 1.572982 |  |  |
| 1-Hexadecanol | 1.850567 |  |  |
| Ethanol | 0.532038 |  |  |
| 5,6,7,-tetrahydropyridol | 0.185057 |  |  |
| 2-Pentadecanol | 0.508906 |  |  |
|  |  |  |  |

Statistical analysis to determine the significance difference between the biomass obtained with Chlorocystis sp. and Picochlorum sp. In Indoor growth system.

|  |  |
| --- | --- |
| Indoor growth comparison | |
| Chlorocystis sp. (g/L) | Picochlorum sp. (g/L) |
| 0.775 | 0.84 |
| 0.74 | 0.816 |
| 0.76 | 0.794 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor | | |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY | |  |  |  |  |  |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| Chlorocystis sp. (g/L) | 3 | 2.275 | 0.758333 | 0.000308 |  |  |
| Picochlorum sp. (g/L) | 3 | 2.45 | 0.816667 | 0.000529 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.005104 | 1 | 0.005104 | 12.18663 | 0.025102 | 7.708647 |
| Within Groups | 0.001675 | 4 | 0.000419 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 0.006779 | 5 |  |  |  |  |

Statistical analysis to determine the significance difference between the Specific growth rate obtained with Chlorocystis sp. and Picochlorum sp. growth system.

|  |  |
| --- | --- |
| Specific growth rate | |
|
| Chlorocystis sp. (1/d) | Picochlorum sp. (1/d) |
| 0.3387 | 0.3970 |
| 0.3478 | 0.3633 |
| 0.3419 | 0.4148 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor | | |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY | |  |  |  |  |  |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| Chlorocystis sp. (1/d) | 3 | 1.028389 | 0.342796 | 2.12E-05 |  |  |
| Picochlorum sp. (1/d) | 3 | 1.175049 | 0.391683 | 0.000685 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.003585 | 1 | 0.003585 | 10.15406 | 0.033326 | 7.708647 |
| Within Groups | 0.001412 | 4 | 0.000353 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 0.004997 | 5 |  |  |  |  |

Statistical analysis to determine the significance difference between the biomass obtained with Chlorocystis sp. and Picochlorum sp. In Outdoor growth system.

|  |  |
| --- | --- |
| Outdoor growth comparison | |
|
| Chlorocystis sp. (g/L) | Picochlorum sp. (g/L) |
| 0.568 | 0.585 |
| 0.581 | 0.569 |
| 0.535 | 0.592 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor | | |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY | |  |  |  |  |  |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| Chlorocystis sp. (g/L) | 3 | 1.684 | 0.561333 | 0.000562 |  |  |
| Picochlorum sp. (g/L) | 3 | 1.746 | 0.582 | 0.000139 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.000641 | 1 | 0.000641 | 1.826996 | 0.247863 | 7.708647 |
| Within Groups | 0.001403 | 4 | 0.000351 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 0.002043 | 5 |  |  |  |  |

Statistical analysis to determine the significance difference between the biomass obtained with Chlorocystis sp. In indoor and outdoor growth system.

|  |  |
| --- | --- |
| Comparison of indoor and outdoor (Chlorocystis sp.) | |
|
| Indoor (g/L) | Outdoor (g/L) |
| 0.775 | 0.568 |
| 0.74 | 0.581 |
| 0.76 | 0.535 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor | | |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY | |  |  |  |  |  |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| Indoor (g/L) | 3 | 2.275 | 0.758333 | 0.000308 |  |  |
| Outdoor (g/L) | 3 | 1.684 | 0.561333 | 0.000562 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.058214 | 1 | 0.058214 | 133.7217 | 0.000319 | 7.708647 |
| Within Groups | 0.001741 | 4 | 0.000435 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 0.059955 | 5 |  |  |  |  |

Statistical analysis to determine the significance difference between the biomass obtained with *Picochlorum* sp.in indoor and outdoor growth system.

|  |  |
| --- | --- |
| Comparison of indoor and outdoor (Picochlorum sp.) | |
|
| Indoor (g/L) | Outdoor (g/L) |
| 0.84 | 0.585 |
| 0.816 | 0.569 |
| 0.794 | 0.592 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor | | |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY | |  |  |  |  |  |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| Indoor (g/L) | 3 | 2.45 | 0.816667 | 0.000529 |  |  |
| Outdoor (g/L) | 3 | 1.746 | 0.582 | 0.000139 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.082603 | 1 | 0.082603 | 247.19 | 0.0000956 | 7.708647 |
| Within Groups | 0.001337 | 4 | 0.000334 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 0.083939 | 5 |  |  |  |  |

Statistical analysis to determine the significance difference between the Biocrude yield obtained with Chlorocystis sp. and Picochlorum sp.

|  |  |
| --- | --- |
| Biocrude yield | |
|
| Chlorocystis sp. (%) | Picochlorum sp. (%) |
| 41.2 | 36.3 |
| 39.4 | 33.9 |
| 38.2 | 37.2 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor | | |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY | |  |  |  |  |  |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| Chlorocystis sp. (%) | 3 | 118.8 | 39.6 | 2.28 |  |  |
| Picochlorum sp. (%) | 3 | 107.4 | 35.8 | 2.91 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 21.66 | 1 | 21.66 | 8.346821 | 0.044604 | 7.708647 |
| Within Groups | 10.38 | 4 | 2.595 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 32.04 | 5 |  |  |  |  |

Statistical analysis to determine the significance difference between the Recycling HTL aqueous phase nutrients obtained with Chlorocystis sp. and Picochlorum sp.

|  |  |
| --- | --- |
| Recycling HTL aqueous phase nutrients | |
|
| Chlorocystis sp. (g/L) | Picochlorum sp. (g/L) |
| 0.785 | 0.8 |
| 0.769 | 0.8138 |
| 0.714 | 0.78585 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Anova: Single Factor | | |  |  |  |  |
|  |  |  |  |  |  |  |
| SUMMARY | |  |  |  |  |  |
| *Groups* | *Count* | *Sum* | *Average* | *Variance* |  |  |
| Chlorocystis sp. (g/L) | 3 | 2.267804 | 0.755935 | 0.001384 |  |  |
| Picochlorum sp. (g/L) | 3 | 2.39965 | 0.799883 | 0.000195 |  |  |
|  |  |  |  |  |  |  |
|  |  |  |  |  |  |  |
| ANOVA |  |  |  |  |  |  |
| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
| Between Groups | 0.002897 | 1 | 0.002897 | 3.668365 | 0.127963 | 7.708647 |
| Within Groups | 0.003159 | 4 | 0.00079 |  |  |  |
|  |  |  |  |  |  |  |
| Total | 0.006056 | 5 |  |  |  |  |

**Comparison of energy consumption in biomass harvesting and HTL process, calorific value of the biocrude oils**

***1. Chlorocystis* sp.**

1.1.Preliminary harvesting

Total culture volume needed to process 1 kg *Chlorocystis* sp. biomass would be = 1/0.68 = 1.782 m3 Considering a pump efficiency of 0.5, the energy requirement for transferring culture from the pond to a harvesting chamber with elevation difference of 2 m (HT1) = 1.782\*9.81\*2/0.5 KJ = 0.069 MJ/kg biomass.

1.2.Secondary harvesting:

Energy requirement for processing primary harvested culture by a centrifuge =5.22 MJ/m3 slurry

50L slurry (2% solid content) secondary harvesting energy requirement (S1) =0.26 MJ

1.3. HTL process:

Energy (kJ) = m\*cp\*∆T

For biomass, m = 1 kg, cp=2 kJ/kg, ∆T = 315 °C

1\*2\*315 = 630 kJ

For Water, m=5.666 kg, cp-4.187, ∆T=315

5.6666\*4.187\*315 = 7472.9 kJ

HTL energy (P1) = 7.4729+0.630 = 8.1029 MJ/Kg

Considering a 50% recovery of heat HT energy consumption= 0.5\* 8.1029 = 4.05 MJ/kg

Total energy needed for harvesting and HTL processing of 1 kg biomass (HT1+S1+P1) = 4.379 MJ/kg.

1.4. The caloric value of the produced biocrude.

The biocrude yield was 34.8% and its corresponding HHV was 32.8 MJ/kg. Hence the calorific value of the produced biocrude oil = 0.348\*32.8 = 11.41MJ/kg biomass

1.5. Energy return on investment (EROI)

The calorific value of the biocrude was divided by the total energy consumption from biomass harvesting to the HTL process.

EROI = 11.41/4.05 = 2.58

***2. Picochlorum* sp.**

**2.1.(experimental scenario ):**

2.1.1. Preliminary harvesting

Total culture volume needed to process 1 kg *Picochlorum* sp. biomass would be = 1/0.582 = 1.7182 m3 Considering a pump efficiency of 0.5, the energy requirement for transferring culture from the pond to a harvesting chamber with elevation difference of 2m (HT2) = 1.7182\*9.81\*2/0.5 KJ = 0.068 MJ/kg biomass.

2.1.2. Secondary harvesting:

Energy requirement for harvesting culture by a centrifuge (S2) = 8.97 MJ/kg

2.1.3. HTL process:

HTL energy (P2) = 4.05 MJ/kg

Total energy needed for harvesting and HTL processing of 1 kg biomass (HT2+S2+P2) = 13.088 MJ/kg

2.1.4. The caloric value of the produced biocrude.

The biocrude yield was 39.6% and its corresponding HHV was 33.4 MJ/kg. Hence the calorific value of the produced biocrude oil = 0.396\*33.4 = 13.23 MJ/kg biomass

2.1.5. Energy return on investment (EROI)

The calorific value of the biocrude was divided by the total energy consumption from biomass harvesting to the HTL process.

EROI = 13.23/13.088 = 1.01

**2.2. (improved scenario):**

2.2.1.Preliminary harvesting:

Total culture volume needed to process 1 kg *Picochlorum* sp. biomass would be = 1/0.582 = 1.7182 m3 Considering a pump efficiency of 0.5, the energy requirement for transferring culture from the pond to a harvesting chamber with elevation difference of 2m (HT3) = 1.7182\*9.81\*2/0.5 KJ = 0.068 MJ/kg biomass.

Energy requirement by cross-flow unit = 3.18 MJ/m3 culture

Energy needed for crossflow filtration = 5.46 MJ/kg

Total energy required for preliminary harvesting = 5.46 + 0.068 = 5.528 MJ/kg

2.2.2.Secondary harvesting:

For 25L slurry secondary harvesting energy requirement (S3)= 0.13 MJ/kg

2.2.3. HTL process:

HTL energy (P3) = 4.05 MJ/kg

Total energy needed for harvesting and HTL processing of 1 kg biomass (HT3+S3+P3) = 9.71 MJ/kg

2.2.4. The caloric value of the produced biocrude.

The biocrude yield was 39.6% and its corresponding HHV was 33.4 MJ/kg. Hence the calorific value of the produced biocrude oil = 0.396\*33.4 = 13.23 MJ/kg biomass

2.1.5. Energy return on investment (EROI)

The calorific value of the biocrude was divided by the total energy consumption from biomass harvesting to the HTL process.

EROI = 13.23/9.71 = 1.36