**Supporting Information**

**Biosynthesis Mechanisms of Medium-Chain Carboxylic Acids and Alcohols in Anaerobic Microalgae Fermentation Regulated by pH Conditions**

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**Text S1 The calculation procedures**

The final concentration of main products including SCCAs, MCCAs and alcohols was converted into COD concentration. The relationship between products and COD is listed in the table below.

**Table** The relationships between products and COD/electron equivalent

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Products | COD value (g COD/g) | Electron equivalent (mol *e*-/mol) | Products | COD value (g COD/g) | Electron equivalent (mol *e*-/mol) |
| Ethanol | 2.09 | 12 | Propionic acid | 1.51 | 14 |
| Propanol | 2.4 | 18 | Butyric acid | 1.82 | 20 |
| Butanol | 2.59 | 24 | Valeric acid | 2.04 | 26 |
| Pentanol | 2.73 | 30 | Caproic acid | 2.21 | 32 |
| Hexanol | 2.82 | 36 | Heptanoic acid | 2.34 | 38 |
| Acetic acid | 1.07 | 8 | Caprylic acid | 2.44 | 44 |

The specificities of primary products are calculated using the following equation:

 (1)

Where *Ci* is the finial concentration (COD g/L) of compound *i*.

To investigate the carbon and electron flow under different pH, the selectivity of each compound at pH 5, 7 and 10 was calculated based on the carbon and electron. Notably, the corresponding product concentrations in the controls were indicated as the utilized substrate stemming from algae for chain elongation and alcohol production. Selectivity is defined as product produced relative to substrates consumed on an electron or carbon basis and is calculated by following equations:

 (2)

 (3)

 (4)

Where *Cinitial*is the concentration of utilized compound *i* (mmol *e*- /L) which is transformed from microalgae biomass, i.e., the concentration of compound *i* in controls. *Cfinal*is the final concentration of compound *i* in the fermentation broth (mmol *e*- /L). Δ*Ci* is the concentration changes of compound *i* (mmol *e*- /L). Similarity, Δ*Ci* is the concentration changes of compound *i* (mmol C /L).

The roles of ethanol were indicated by calculating the transfer efficiency from ethanol to corresponding products according to the previous study [18]. Ethanol was used to 1) produce acetate via RBO, 2) perform chain elongation as electron donor via RBO, 3) conduct excessive ethanol oxidation (EEO) and 4) provide reducing power to transform carboxylic acid into corresponding alcohols.

Total ethanol consumption (mmol/L):

 (5)

Ethanol used for chain elongation (mmol/L):

 (6)

Ethanol used for alcohol production (mmol/L):

 (7)

Ethanol used for producing acetate via RBO (mmol/L):

 (8)

 (9)

Where *Cf* is the final concentration of ethanol in the fermentation broth (mmol/L). *Ci* is the initial concentration of ethanol that added in the reactors (mmol/L). Δ*i* is the concentration changes of compound *i* (mmol/L).

**Text S2 The calculation of undissociated MCCA concentrations in E5 and E7 groups**

For any organic acid, there is a chemical equilibrium reaction in the aqueous solution:

Therefore, the dissociation constant is:

 (10)

Then

 (11)

**Table S1** The average concentration (mg-COD/L) of main products and their selectivity (electron/ carbon) for the microalgae fermentation reactor during the steady stage (mean ± standard error). If a concentration was below the detection limit, then the concentration was represented as 0.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Group | | C5 | E5 | C7 | E7 | C10 | E10 |
| pH | | 5 | | 7 | | 10 | |
| Ethanol residue | | 0 | 3,724.86 ± 853.10 | 0 | 0 | 0 | 3,278.05 ± 1424.08 |
| SCCAs | C2 | 816.25 ± 188.26 | 132.81 ± 93.31 | 1,454.79 ± 350.86 | 93.08 ± 76.86 | 1,315.15 ± 184.06 | 6,223.06 ± 772.89 |
| C3 | 171.99 ± 43.56 | 0 | 567.36 ± 126.69 | 447.20 ± 123.98 | 232.36 ± 50.01 | 209.97 ± 54.00 |
| C4 | 472.61 ± 123.16 | 4,380.34 ± 342.23 | 376.57 ± 93.76 | 6,974.01 ± 731.25 | 454.08 ± 141.81 | 284.62 ± 98.68 |
| C5 | 310.25 ± 95.41 | 288.54 ± 92.96 | 314.87 ± 107.88 | 769.97 ± 133.86 | 360.20 ± 114.84 | 353.58 ± 132.94 |
| MCCAs | C6 | 94.03 ± 37.90 | 1,034.78 ± 171.134 | 110.92 ± 48.40 | 7,416.55 ± 859.24 | 43.76 ± 21.17 | 42.57 ± 18.14 |
| C7 | 0 | 0 | 0 | 16.02 ± 6.34 | 0 | 0 |
| C8 | 0 | 0 | 0 | 42.06 ± 23.33 | 0 | 0 |
| Alcohols | C3 | 0 | 487.69 ± 197.58 | 0 | 453.95 ± 79.41 | 0 | 0 |
| C4 | 0 | 2,214.33 ± 251.28 | 0 | 599.44 ± 81.51 | 0 | 0 |
| C5 | 0 | 26.19 ± 13.17 | 0 | 31.33 ± 4.65 | 0 | 0 |
| C6 | 0 | 84.10 ± 46.23 | 0 | 172.49 ± 33.70 | 0 | 0 |
| Caproate selectivity | | - | 6.78%/ 7.42% | - | 40.13%/ 43.44% | - | - |
| Heptanoate selectivity | | - | - | - | 0.09%/ 0.09% | - | - |
| Caprylate selectivity | | - | - | - | 0.23%/ 0.24% | - | - |
| Butanol selectivity | | - | 16.01%/ 15.57% | - | 3.30%/ 3.18% | - | - |
| Hexanol selectivity | | - | 0.61%/ 0.59% | - | 0.95%/ 0.91% | - | - |
| Ethanol use for elongation of fatty acids through the RBO | | - | 23.47% | - | 59.27% | - | - |
| Ethanol use for alcohol production | | - | 22.99% | - | 6.72% | - | - |
| Other unedified Ethanal oxidation | | - | 30.32% | - | 1.02% | - | 100% |

**Table S2** The specificities of primary products (on a COD basis) for each group

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Group | C5 | E5 | C7 | E7 | C10 | E10 |
| Propanol | 0.00% | 5.63% | 0.00% | 2.67% | 0.00% | 0.00% |
| Butanol | 0.00% | 25.58% | 0.00% | 3.52% | 0.00% | 0.00% |
| Pentanol | 0.00% | 0.30% | 0.00% | 0.18% | 0.00% | 0.00% |
| Hexanol | 0.00% | 0.97% | 0.00% | 1.01% | 0.00% | 0.00% |
| Acetate | 43.72% | 1.53% | 51.50% | 0.55% | 54.60% | 87.43% |
| Propionate | 9.21% | 0.00% | 20.09% | 2.63% | 9.65% | 2.95% |
| Butyrate | 25.32% | 50.61% | 13.33% | 40.96% | 18.85% | 4.00% |
| Valerate | 16.62% | 3.33% | 11.15% | 4.52% | 14.95% | 4.97% |
| Caproate | 5.04% | 11.96% | 3.93% | 43.56% | 1.82% | 0.60% |
| Heptanoate | 0.00% | 0.00% | 0.00% | 0.09% | 0.00% | 0.00% |
| Caprylate | 0.00% | 0.00% | 0.00% | 0.25% | 0.00% | 0.00% |
| H2 | 0.09% | 0.08% | 0.00% | 0.06% | 0.13% | 0.05% |

**Table S3** The selectivity of each compound at pH 5 based on the electron and carbon

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | Initial con.  (mM) | Final con.  (mM) | Δ con.  (mM) | Selectivity mol *e*- % | Selectivity mol C % |
| Ethanol | 173.98 | 38.74 | -135.23 | N.A. | N.A. |
| Propanol | 0 | 3.39 | 3.39 | 3.52% | 3.42% |
| Butanol | 0 | 11.55 | 11.55 | 16.01% | 15.57% |
| Pentanol | 0 | 0.11 | 0.11 | 0.19% | 0.18% |
| Hexanol | 0 | 0.29 | 0.29 | 0.61% | 0.59% |
| Acetate | 12.71 | 2.07 | -10.65 | N.A. | N.A. |
| Propionate | 1.54 | 0.00 | -1.54 | N.A. | N.A. |
| Butyrate | 2.95 | 27.35 | 24.40 | 28.17% | 32.87% |
| Valerate | 1.49 | 1.39 | -0.10 | N.A. | N.A. |
| Caproate | 0.37 | 4.04 | 3.67 | 6.78% | 7.42% |
| Heptanoate | 0 | 0 | 0 | 0% | 0% |
| Caprylate | 0 | 0 | 0 | 0% | 0% |
| CH4 | <1% | <1% | N.A. | N.A. | N.A. |
| CO2 | 0.58 | 0.34 | -0.24 | N.A. | N.A. |
| H2 | 0.21 | 0.85 | 0.64 | N.A. | N.A. |
| Unidentified |  |  |  | 44.73% | 39.95% |

**Table S4** The selectivity of each compound at pH 7 based on the electron and carbon

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | Initial con.  (mM) | Final con.  (mM) | Δ con.  (mM) | Selectivity mol e- % | Selectivity mol C % |
| Ethanol | 173.98 | 0 | -173.98 | N.A. | N.A. |
| Propanol | 0 | 3.15 | 3.15 | 2.50% | 2.40% |
| Butanol | 0 | 3.13 | 3.13 | 3.30% | 3.18% |
| Pentanol | 0 | 0.13 | 0.13 | 0.17% | 0.17% |
| Hexanol | 0 | 0.60 | 0.60 | 0.95% | 0.91% |
| Acetate | 22.66 | 1.45 | -21.21 | N.A. | N.A. |
| Propionate | 5.08 | 4.00 | -1.08 | N.A. | N.A. |
| Butyrate | 2.35 | 43.54 | 41.19 | 36.25% | 41.86% |
| Valerate | 1.51 | 3.70 | 2.19 | 2.50% | 2.78% |
| Caproate | 0.43 | 28.93 | 28.50 | 40.13% | 43.44% |
| Heptanoate | 0 | 0.05 | 0.05 | 0.09% | 0.09% |
| Caprylate | 0 | 0.12 | 0.12 | 0.23% | 0.24% |
| CH4 | <1% | <1% | N.A. | N.A. | N.A. |
| CO2 | 0.66 | 0.19 | N.A. | N.A. | N.A. |
| H2 | 0.01 | 1.25 | N.A. | N.A. | N.A. |
| Unidentified |  |  |  | 13.87% | 4.92% |

**Table S5** The selectivity of each compound at pH 10 based on the electron and carbon

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Compound | Initial con.  (mM) | Final con.  (mM) | Δ con.  (mM) | Selectivity mol e- % | Selectivity mol C % |
| Ethanol | 173.98 | 31.89 | -142.09 | N.A. | N.A. |
| Propanol | 0 | 0 | 0 | 0 | 0 |
| Butanol | 0 | 0 | 0 | 0 | 0 |
| Pentanol | 0 | 0 | 0 | 0 | 0 |
| Hexanol | 0 | 0 | 0 | 0 | 0 |
| Acetate | 20.49 | 96.93 | 76.45 | 35.35% | 52.87% |
| Propionate | 2.08 | 1.88 | -0.20 | N.A. | N.A. |
| Butyrate | 2.84 | 1.78 | -1.06 | N.A. | N.A. |
| Valerate | 1.73 | 1.70 | -0.03 | N.A. | N.A. |
| Caproate | 0.17 | 0.17 | 0.00 | N.A. | N.A. |
| Heptanoate | 0 | 0 | 0 | 0 | 0 |
| Caprylate | 0 | 0 | 0 | 0 | 0 |
| CH4 | <1% | <1% | N.A. | N.A. | N.A. |
| CO2 | <1% | <1% | N.A. | N.A. | N.A. |
| H2 | 0.38 | 0.47 | 0.09 | N.A. | N.A. |
| Unidentified |  |  |  | 64.65% | 48.66% |

**Table S6** The overall details of metagenomic assembly results

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sample | Read length (bp) | Metagenomic raw data | | Metagenomic clean data | | Assembly and predicted results | | | | | |
| Raw reads | Raw base (bp) | Clean reads | Clean bases (bp) | Contigs | Contigs bases (bp) | N50 (bp) | ORFs | Total ORFs length | Average ORFs length |
| C7 | 150 | 44,446,366 | 6,711,401,266 | 43,917,120 | 6,589,526,274 | 555,278 | 358,024,509 | 657 | 720,727 | 318,398,805 | 441.77 |
| E5 | 46,135,516 | 6,966,462,916 | 45,559,734 | 6,796,213,934 | 655,223 | 436,024,171 | 689 | 864,149 | 390,091,629 | 451.42 |
| E7 | 43,868,618 | 6,624,161,318 | 43,459,070 | 6,513,041,058 | 617,805 | 411,751,374 | 699 | 817,592 | 368,064,114 | 450.18 |
| E10 | 40,842,952 | 6,167,285,752 | 40,395,430 | 6,053,848,787 | 539,639 | 379,094,270 | 758 | 726,659 | 338,560,167 | 465.91 |