**Revised**

**Supplementary Material**

**Hydrogen from dark fermentation of the organic fraction of waste diapers: optimization based on response surface experiments**

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**Table SM1.** Coded levels of the factors in the response surface experiment

|  |  |  |  |
| --- | --- | --- | --- |
| **Coded levels** | ***C/N*a**  **(gC/gN)** | ***TSi*b**  **(%)** | ***T*c**  **(oC)** |
| -αd | 27 | 12 | 31 |
| -1 | 30 | 15 | 37 |
| 0 | 35 | 20 | 46 |
| 1 | 40 | 25 | 55 |
| αe | 43 | 28 | 61 |

acarbon-to-nitrogen ratio of the feedstock; b initial total solids in the feedstock; ctemperature of operation; d-α = -1.68179; eα = 1.68179

**Table SM2.** Experimental design layout and main results

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Std Order | Run order | *C/N*a  (gC/gN) | *TSi*b  (%) | *T*c  (°C) | Initial pH  (-) | *Y’H*2  Cumulative production of H2 or yield  (mmol H2/g*TSi*) | *∑VOA*  (mg COD/kgdb) | *∑SOLV*  (mg COD/kgdb) |
| 33 | 1 | 35 | 20 | 31 | 6.80 | 0.8615 | 9105.33 | 66.57 |
| 24 | 2 | 40 | 25 | 37 | 6.73 | 1.2617 | 4079.73 | 57.60 |
| 9 | 3 | 27 | 20 | 46 | 6.60 | 0.5795 | 2211.05 | 9.01 |
| 28 | 4 | 40 | 25 | 55 | 6.40 | 0.2996 | 2747.92 | 32.28 |
| 27 | 5 | 30 | 25 | 55 | 6.56 | 0.7116 | 481.96 | 50.54 |
| 5 | 6 | 30 | 15 | 55 | 6.86 | 0.4516 | 1363.37 | 45.52 |
| 15 | 7 | 35 | 20 | 46 | 6.70 | 1.0308 | 6056.06 | 49.05 |
| 34 | 8 | 35 | 20 | 61 | 6.76 | 0.1188 | 1422.76 | 13.20 |
| 35 | 9 | 35 | 20 | 46 | 6.83 | 1.2650 | 968.54 | 198.76 |
| 4 | 10 | 40 | 25 | 37 | 6.59 | 0.7693 | 1422.18 | 167.50 |
| 23 | 11 | 30 | 25 | 37 | 6.63 | 0.6413 | 96.22 | 77.89 |
| 32 | 12 | 35 | 28 | 46 | 6.95 | 0.2578 | 1029.35 | 137.78 |
| 18 | 13 | 35 | 20 | 46 | 6.73 | 1.1667 | 1045.39 | 0.10 |
| 25 | 14 | 30 | 15 | 55 | 6.89 | 0.6331 | 1588.33 | 41.20 |
| 37 | 15 | 35 | 20 | 46 | 6.76 | 0.9520 | 1094.54 | 12.61 |
| 31 | 16 | 35 | 12 | 46 | 6.89 | 1.7729 | 1980.80 | 29.36 |
| 19 | 17 | 35 | 20 | 46 | 6.65 | 0.9286 | 438.32 | 0.10 |
| 30 | 18 | 43 | 20 | 46 | 6.54 | 1.1306 | 872.09 | 13.27 |
| 7 | 19 | 30 | 25 | 55 | 6.63 | 0.3490 | 833.98 | 39.42 |
| 12 | 20 | 35 | 28 | 46 | 6.64 | 0.3675 | 426.43 | 58.55 |
| 6 | 21 | 40 | 15 | 55 | 6.82 | 0.8267 | 889.18 | 40.19 |
| 26 | 22 | 40 | 15 | 55 | 6.95 | 0.7272 | 1002.24 | 43.02 |
| 29 | 23 | 27 | 20 | 46 | 6.58 | 0.2633 | 653.25 | 34.75 |
| 11 | 24 | 35 | 12 | 46 | 6.91 | 0.9282 | 1291.21 | 58.84 |
| 22 | 25 | 40 | 15 | 37 | 6.97 | 1.9755 | 683.39 | 24.47 |
| 36 | 26 | 35 | 20 | 46 | 6.98 | 1.2459 | 654.06 | 238.70 |
| 38 | 27 | 35 | 20 | 46 | 6.95 | 1.0741 | 822.66 | 181.73 |
| 10 | 28 | 43 | 20 | 46 | 6.95 | 0.3555 | 776.19 | 27.64 |
| 8 | 29 | 40 | 25 | 55 | 6.75 | 0.5787 | 422.46 | 13.13 |
| 13 | 30 | 35 | 20 | 31 | 6.91 | 1.2424 | 590.99 | 30.73 |
| 21 | 31 | 30 | 15 | 37 | 6.90 | 0.6654 | 831.38 | 34.12 |
| 14 | 32 | 35 | 20 | 61 | 6.81 | 1.0707 | 743.44 | 23.06 |
| 16 | 33 | 35 | 20 | 46 | 6.82 | 1.5003 | 735.83 | 64.20 |
| 2 | 34 | 40 | 15 | 37 | 6.94 | 2.4760 | 1329.11 | 24.77 |
| 39 | 35 | 35 | 20 | 46 | 6.87 | 0.7078 | 685.44 | 16.39 |
| 1 | 36 | 30 | 15 | 37 | 6.75 | 0.6200 | 1003.59 | 22.31 |
| 17 | 37 | 35 | 20 | 46 | 6.78 | 1.6165 | 554.93 | 21.02 |
| 40 | 38 | 35 | 20 | 46 | 6.74 | 0.6717 | 525.61 | 15.73 |
| 3 | 39 | 30 | 25 | 37 | 6.72 | 0.6204 | 257.07 | 14.83 |
| 20 | 40 | 35 | 20 | 46 | 6.89 | 1.0015 | 502.91 | 22.39 |

acarbon-to-nitrogen ratio of the feedstock; b initial total solids in the feedstock; ctemperature of operation

**Table SM3.** Analysis of variance for the response surface regression *Y’H2*vs *C/N*, *TSi*, *T*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Degrees of**  **Freedom** | **Sum of squares** | **Mean of the Sum of Squares** | **Fexpa** | ***p* value** |  |
| Model | 9 | 6.3702 | 0.70780 | 6.58 | 0.000 |  |
| Linear | 3 | 3.9614 | 1.302048 | 12.27 | 0.000 |  |
| CN | 1 | 1.0511 | 1.050106 | 9.77 | 0.004 |  |
| ST | 1 | 1.5932 | 1.59316 | 14.81 | 0.001 |  |
| T | 1 | 1.3172 | 1.31722 | 12.24 | 0.001 |  |
| Squares | 3 | 0.8059 | 0.26863 | 2.50 | 0.079 |  |
| CN\*CN | 1 | 0.6533 | 0.65326 | 6.07 | 0.020 |  |
| TS\*TS | 1 | 0.1043 | 0.10431 | 0.97 | 0.333 |  |
| T\*T | 1 | 0.1200 | 0.12001 | 1.12 | 0.299 |  |
| 2-factor interaction | 3 | 1.6029 | 0.53430 | 4.97 | 0.006 |  |
| CN\*TS | 1 | 0.5808 | 0.58078 | 5.40 | 0.027 |  |
| CN\*T | 1 | 0.8319 | 0.83193 | 7.73 | 0.009 |  |
| TS\*T | 1 | 0.1902 | 0.19019 | 1.77 | 0.194 |  |
| Error | 30 | 3.2279 | 0.10760 |  |  |  |
| Lack-of-Fit | 5 | 0.7317 | 0.14634 | 1.47 | 0.236 |  |
| Pure error | 25 | 2.4962 | 0.09985 |  |  |  |
| Total | 39 | 9.5981 |  |  |  |  |
| **Model Summary** | | | | | | |
| **S b** | **R2 c** | **Adjusted R2** | **Predicted R2** |  |  |  |
| 0.33 | 66.3 % | 56.28% | 37.36% |  |  |  |
| **Coded Coefficients** | | | | | | |
| **Term** | **Effect** | **Coefficient** | **Standard error** | **T value** | **P value** | **VIFd** |
| Constant |  | 1.0861 | 0.0942 | 11.53 | 0.000 |  |
| CN | 0.640 | 0.320 | 0.102 | 3.13 | 0.004 | 1.00 |
| TS | -0.788 | -0.394 | 0.102 | -3.85 | 0.004 | 1.00 |
| T | -0.735 | -0.367 | 0.105 | -3.50 | 0.001 | 1.00 |
| CN\*CN | -0.831 | -0.415 | 0.169 | -2.46 | 0.020 | 1.01 |
| TS\*TS | -0.332 | -0.166 | 0.169 | -0.98 | 0.333 | 1.01 |
| T\*T | -0.362 | -0.181 | 0.172 | -1.06 | 0.299 | 1.01 |
| CN\*TS | -0.975 | -0.488 | 0.210 | -2.32 | 0.027 | 1.00 |
| CN\*T | -1.216 | -0.608 | 0.219 | -2.7 | 0.009 | 1.00 |
| TS\*T | 0.851 | 0.291 | 0.219 | 1.33 | 0.194 | 1.00 |
| **Predictions and diagnostics for outliers** | | | | | | |
| **Observation** | **Experimental PH2** | **Predicted**  **PH2** | **Residual** | **Standardized Residual** |  |  |
| 28 | 0.355 | 0.991 | -0.635 | -2.30 Re |  |  |
|  | | | | | | |

avalue of the Fisher statistic based on experimental results; bstandard deviation of model; ccorrelation coefficient of model; dVIF Inflation factor of the variance, eR large residue

**Table SM4.** Analysis of variance for the response surface regression: sum of solvents *ΣSOLV* vs *C/N*, *TSi*, and *T*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Source** | **Degrees of**  **Freedom** | **Sum of squares** | **Mean of the Sum of Squares** | **Fexpa** | **P value** |  |
| **Linear model** | 9 | 23188 | 2576.5 | 0.79 | 0.624 |  |
| 3 | 6669 | 2223.1 | 0.69 | 0.568 |  |
| C/N | 1 | 201 | 200.8 | 0.06 | 0.805 |  |
| TS | 1 | 4685 | 4684.7 | 1.44 | 0.239 |  |
| T | 1 | 1784 | 1783.7 | 0.55 | 0.464 |  |
| Square | 3 | 10231 | 3410.5 | 1.05 | 0.384 |  |
| CN\*CN | 1 | 6909 | 6909.3 | 2.13 | 0.155 |  |
| TS\*TS | 1 | 199 | 199.1 | 0.06 | 0.806 |  |
| T\*T | 1 | 3472 | 3471.1 | 1.07 | 0.309 |  |
| 2- Way Interaction | 3 | 6288 | 2095.9 | 0.65 | 0.591 |  |
| CN\*TS | 1 | 607 | 606.8 | 0.19 | 0.668 |  |
| CN\*T | 1 | 1876 | 1875.8 | 0.58 | 0.453 |  |
| TS\*T | 1 | 3805 | 3805.1 | 1.17 | 0.287 |  |
| Error | 30 | 97307 | 3243.6 |  |  |  |
| Lack-of-Fit | 5 | 2736 | 547.3 | 0.140 | 0.980 |  |
| Pure error | 25 | 94571 | 3782.8 |  |  |  |
| Total | 39 | 120496 |  |  |  |  |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Model summary** | | | | | | |
| **Sb** | **R2 c** | **Adjusted R2** | **Predicted R2** |  |  |  |
| 56.95 | 23.19% | 7.95% | 0.00% |  |  |  |
| **Coded Coefficients** | | | | | | |
| **Term** | **Effect** | **Coefficient** | **Standard error** | **T value** | **P value** | **VIFd** |
| Constant |  | 67.9 | 16.4 | 4.15 | 0.000 |  |
| CN | 8.9 | 4.4 | 17.8 | 0.25 | 0.805 | 1.00 |
| TS | 42.8 | 21.4 | 17.8 | 1.20 | 0.239 | 1.00 |
| T | -27.0 | -13.5 | 18.2 | -0.74 | 0.464 | 1.00 |
| CN\*CN | -85.4 | -42.7 | 29.3 | -1.46 | 0.155 | 1.01 |
| TS\*TS | 14.5 | 7.3 | 29.3 | 0.25 | 0.806 | 1.01 |
| T\*T | -61.1 | -30.8 | 29.8 | -1.03 | 0.309 | 1.01 |
| CN\*TS | 31.5 | 15.8 | 36.4 | 0.43 | 0.668 | 1.00 |
| CN\*T | -57.7 | -28.9 | 38.0 | -0.76 | 0.453 | 1.00 |
| TS\*T | -82.2 | -41.1 | 38.0 | -1.08 | 0.287 | 1.00 |
| Predictions and diagnostics for outliers | | | | | | |
| Observation | Experimental  ∑SOLV | Predicted ΣSOLV | Residual | Standardized Residual |  |  |
| 9 | 198.8 | 67.9 | 130.8 | 2.4. Re |  |  |
| 26 | 238.7 | 67.9 | 170.8 | 3.13 R |  |  |
| 27 | 181.7 | 67.9 | 113.8 | 2.09 R |  |  |

avalue of the Fisher statistic based on experimental results; bstandard deviation of model; ccorrelation coefficient of model; dVIF Inflation factor of the variance, eR large residue

**Table SM5.** Optimization of the response for sum of solvents

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Response variable | Goal | Lower | Upper | Weight | Importance |
| **∑SOLV**a | Minimizar | 0 | 238.71 | 1 | 1 |
| **Solution** | | | | | |
| **Solution** | **C/N**b | **TSi**c | **T**d | **Predicted**  **∑SOLV** | **Composite Desirability** |
| 1 | 27 | 12 | 31 | -64.88 | 1 |
| **Response** | **Predicted value** | **Standard error** | **95% confidence interval)** | **95%**  **prediction interval)** |  |
| **∑SOLV** | **-64.9** | 83.1 | (-234.5,104.8) | (-270.6,140.8) |  |

asum of solvents; bcarbon-to-nitrogen ratio of the feedstock; cinitial total solids in the feedstock; dtemperature of operation

The R2 statistic for the analysis of variance of ΣSOLV explained 23.19% of the experimental variation, which is also a poor value whereas the adjusted R2 was only 7.95% with a standard deviation of the residuals of 8.69. The P values greater than 0.05 indicate that there is no significant difference in this response, except for the amount of TSi (Table SM4).

**Table SM6.** Composition of organic fraction of waste diapers (*OFWD*)

|  |  |
| --- | --- |
| **Parameter** | ***OFWD*** |
| pH | 6.81 ± 0.16 |
| Alkalinity (mg CaCO3/kg) | 844 ± 41 |
| VS (% TS) | 84.82 ± 1.41 |
| Ashes (% TS) | 15.12 ± 1.65 |
| TKN (% TS) | 0.47 ± 0.04 |
| C/N | 104.67±1.05 |
| Cellulose (% VS) | 60.95±3.24 |
| Hemicellulose (% VS) | 12.54± 3.61 |
| Lignin (% VS) | 8.79± 0.75 |
| Soluble COD (mg/L) | 1840 ± 423 |
| Protein (% VS) | 2.81 ± 0.12 |

**Table SM7**. Adjustment of Carbon-Nitrogen ratio

|  |  |  |
| --- | --- | --- |
| ***C/N***  **(required)** | ***OFWD***  **(g)** | **NH4Cl**  **(g)** |
| 27 | 0.96 | 0.04 |
| 30 | 0.97 | 0.03 |
| 35 | 0.98 | 0.02 |
| 40 | 0.98 | 0.02 |
| 43 | 0.99 | 0.01 |

**Table SM8.** Values of coefficients in the equation of the response hypersurface of acetic acid concentration

|  |  |
| --- | --- |
| *Coefficient* | *Estimation* |
| constant | -2111.15 |
| A:C:Na | 636.30 |
| B:Temperature | -302.77 |
| C:TSib | -102.21 |
| AA | -10.41 |
| AB | -2.61 |
| AC | 12.17 |
| BB | 4.51 |
| BC | -2.48 |
| CC | -5.38 |

acarbon-to-nitrogen ratio of the feedstock; b initial total solids in the feedstock

The values of the factors should be introduced in the Eq. 6 in their natural values-units.

**Table SM9.** Values of coefficients in the equation of the response hypersurface of lactic acid concentration

|  |  |
| --- | --- |
| *Coefficient* | *Estimation* |
| constant | -24801.20 |
| A:C:Na | 4065.52 |
| B:Temperature | -2157.16 |
| C:TSib | 2541.52 |
| AA | -70.30 |
| AB | -4.79 |
| AC | 43.40 |
| BB | 20.96 |
| BC | -3.82 |
| CC | -99.16 |

acarbon-to-nitrogen ratio of the feedstock; b initial total solids in the feedstock

The values of the factors should be introduced in the corresponding equation in their natural values-units (not standardized).

**Table SM10.** Pearson correlation matrix

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | ***C/N*a** | ***HAc* b** | ***HBu*c** | ***HLac*d** | ***HPr*e** | **Final pH** | **Ratio *A:B*f** | ***TSi*g** | **Temperature** |
| ***C/N*** | 1.0000 | 0.1736k | 0.0343 | -0.1273 | 0.0134 | 0.2283 | 0.3329 | 0.0000 | 0.0000 |
|  |  | (15)i | (15) | (15) | (15) | (15) | (15) | (15) | (15) |
|  |  | 0.5361j | 0.9033 | 0.6512 | 0.9623 | 0.4132 | 0.2254 | 1.0000 | 1.0000 |
| ***HAc*** | 0.1736 | 1.0000 | ***0.9023*** | ***0.8341*** | ***0.4323*** | 0.0323 | 0.2375 | -0.0325 | -0.3038 |
|  | (15) |  | (15) | (15) | (15) | (15) | (15) | (15) | (15) |
|  | 0.5361 |  | ***0.0000*** | ***0.0001*** | ***0.1075*** | 0.9090 | 0.3941 | 0.9083 | 0.2710 |
| ***HBu*** | 0.0343 | 0.9023 | 1.0000 | ***0.9071*** | ***0.4166*** | 0.0543 | -0.1990 | -0.0927 | ***-0.4583*** |
|  | (15) | (15) |  | (15) | (15) | (15) | (15) | (15) | (15) |
|  | 0.9033 | 0.0000 |  | ***0.0000*** | ***0.1224*** | 0.8477 | 0.4770 | 0.7424 | ***0.0857*** |
| ***HLac*** | -0.1273 | 0.8341 | 0.9071 | 1.0000 | ***0.6080*** | -0.0256 | -0.1550 | -0.0495 | ***-0.5285*** |
|  | (15) | (15) | (15) |  | (15) | (15) | (15) | (15) | (15) |
|  | 0.6512 | 0.0001 | 0.0000 |  | ***0.0162*** | 0.9278 | 0.5812 | 0.8608 | ***0.0428*** |
| ***HPr*** | 0.0134 | 0.4323 | 0.4166 | 0.6080 | 1.0000 | -0.0388 | 0.0632 | -0.0368 | -0.4890 |
|  | (15) | (15) | (15) | (15) |  | (15) | (15) | (15) | (15) |
|  | 0.9623 | 0.1075 | 0.1224 | 0.0162 |  | 0.8909 | 0.8230 | 0.8965 | 0.0643 |
| **Final pH** | 0.2283 | 0.0323 | 0.0543 | -0.0256 | -0.0388 | 1.0000 | -0.1414 | ***0.8858*** | 0.0386 |
|  | (15) | (15) | (15) | (15) | (15) |  | (15) | (15) | (15) |
|  | 0.4132 | 0.9090 | 0.8477 | 0.9278 | 0.8909 |  | 0.6153 | ***0.0000*** | 0.8913 |
| **Ratio *A:B*** | 0.3329 | 0.2375 | -0.1990 | -0.1550 | 0.0632 | -0.1414 | 1.0000 | 0.2315 | 0.3167 |
|  | (15) | (15) | (15) | (15) | (15) | (15) |  | (15) | (15) |
|  | 0.2254 | 0.3941 | 0.4770 | 0.5812 | 0.8230 | 0.6153 |  | 0.4065 | 0.2502 |
| ***TSi*** | 0.0000 | -0.0325 | -0.0927 | -0.0495 | -0.0368 | -0.8858 | 0.2315 | 1.0000 | 0.0000 |
|  | (15) | (15) | (15) | (15) | (15) | (15) | (15) |  | (15) |
|  | 1.0000 | 0.9083 | 0.7424 | 0.8608 | 0.8965 | 0.0000 | 0.4065 |  | 1.0000 |
| **Temperature** | 0.0000 | -0.3038 | -0.4583 | -0.5285 | -0.4890 | 0.0386 | 0.3167 | 0.0000 | 1.0000 |
|  | (15) | (15) | (15) | (15) | (15) | (15) | (15) | (15) |  |
|  | 1.0000 | 0.2710 | 0.0857 | 0.0428 | 0.0643 | 0.8913 | 0.2502 | 1.0000 |  |

a carbon-to-nitrogen ratio; bAcetic acid; cButiricacid; dLactic acid; ePropionic acid; facteic to butyric acid ratio; ginitial initial total solids in the feedstock**;** hCorrelation coefficient; i sample size; jp-value of the correlation coefficient estimation

**Table SM11.** Coordinates of objects in the *PC1-PC2-PC3* space

|  |  |  |  |
| --- | --- | --- | --- |
| *Objects* | *Component*  *1* | *Component*  *2* | *Component*  *3* |
| 1 | 6.08 | -0.36 | -0.027 |
| 2 | 1.21 | -2.02 | 1.68 |
| 3 | 0.72 | -0.38 | -1.98 |
| 4 | -0.83 | -1.72 | 1.88 |
| 5 | -1.43 | -1.59 | -1.15 |
| 6 | -0.79 | 0.89 | 0.49 |
| 7 | -0.037 | 0.73 | -0.28 |
| 8 | -1.33 | 0.22 | 0.89 |
| 9 | -1.20 | -1.51 | -1.63 |
| 10 | -0.98 | -1.51 | -0.41 |
| 11 | 0.25 | 2.07 | 1.02 |
| 12 | -0.89 | 0.57 | 1.04 |
| 13 | -0.91 | 1.50 | 0.63 |
| 14 | 0.10 | 1.73 | -0.41 |
| 15 | 0.039 | 1.36 | -1.74 |

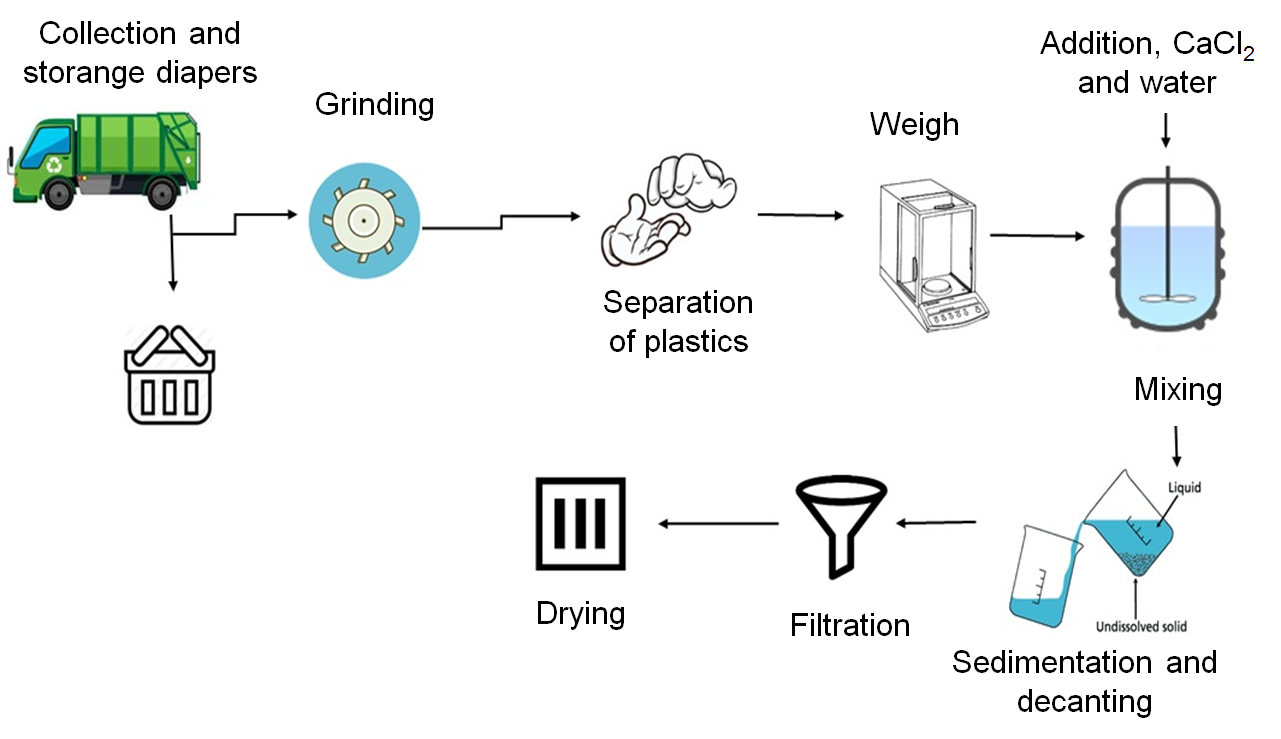
**Table SM12.** Summary of the groups of treatments according to dendrogram Fig. 6

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Treat-  ment | *C/N* (gC/gN) | *TSi*  (%) | *T*  (°C) | *HAc*  (mg *COD*/  kgTS) | *HPr*  (mg *COD*/  kgTS) | *HBu*  (mg *COD*/  kgTS) | *HLac*  (mg *COD*/kgTS) | Ratio *A/B*  (mg *COD*-*HAc* /  mg *COD*-*HBu*) | Final pH  (-) |
| 1 | 0.00 | 0.00 | -1.69 | 3.24 | 1.31 | 3.58 | 3.22 | -0.39 | -0.17 |
| 2 | 1.03 | 1.03 | -1.02 | 0.54 | 2.33 | -0.28 | 0.21 | 2.26 | -0.97 |
| 3 | -1.65 | 0.00 | 0.00 | -0.58 | 1.34 | -0.16 | 1.03 | -0.74 | -0.59 |
| 4 | 1.03 | 1.03 | 1.02 | 0.39 | -0.42 | -0.28 | -0.34 | 1.88 | -1.06 |
| 11 | 0.00 | -1.65 | 0.00 | 0.45 | -0.44 | -0.12 | 0.36 | 1.14 | 1.75 |
|  |  |  |  |  |  |  |  |  |  |
| 5 | -1.03 | 1.03 | 1.02 | -0.53 | -0.78 | -0.34 | -0.58 | -0.26 | -1.58 |
| 9 | -1.03 | 1.03 | -1.02 | -0.86 | -1.29 | -0.64 | -0.53 | -0.04 | -1.40 |
| 10 | 0.00 | 1.65 | 0.00 | -0.50 | -0.67 | -0.40 | -0.39 | 0.11 | -0.75 |
|  |  |  |  |  |  |  |  |  |  |
| 6 | -1.03 | -1.03 | 1.02 | 0.22 | -0.33 | -0.23 | -0.59 | 1.21 | 0.67 |
| 7 | 0.00 | 0.00 | 0.00 | -0.25 | 0.24 | -0.04 | -0.10 | -0.31 | 0.87 |
| 8 | 0.00 | 0.00 | 1.69 | -0.14 | -0.53 | -0.34 | -0.54 | 0.88 | 0.41 |
| 12 | 1.65 | 0.00 | 0.00 | -0.22 | -1.05 | -0.25 | -0.59 | 0.25 | 0.50 |
| 13 | 1.03 | -1.03 | 1.02 | -0.37 | -0.40 | -0.19 | -0.51 | -0.26 | 0.75 |
| 14 | 1.03 | -1.03 | -1.02 | -0.67 | 0.40 | -0.08 | -0.43 | -0.97 | 0.95 |
| 15 | -1.03 | -1.03 | -1.02 | -0.72 | 0.30 | -0.23 | -0.24 | -0.93 | 0.63 |

**Appendix 2. Figures**

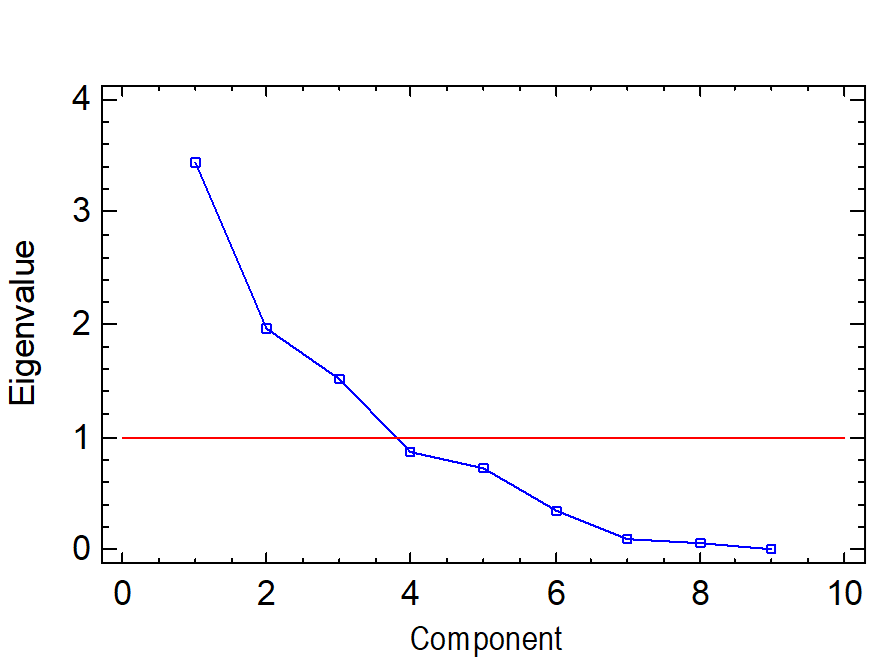
**List of Figures**

|  |  |
| --- | --- |
| No. | Figure legend |
| SM1 | Separation process for the organic fraction of waste diapers |
| SM2 | Contour plots of key organic acids of the response surface experiment: (a) acetic acid; (b) lactic acid |
| SM3 | Scree plot |

 **Fig. SM1.** Separation process for the organic fraction of waste diapers

|  |  |
| --- | --- |
|  |  |

**Fig. SM2.** Contour plots of key organic acids of the response surface experiment: (a) acetic acid; (b) lactic acid



**Fig. SM3.** Scree plot

**Appendix 3. Ratio A/B**

The ratio A/B acetic-to-butyric acid has been used by other researchers in the study of hydrogenogenic fermentation performance (Chang and Lin, 2004; Robledo-Narváez et al., 2013; Kim et al., 2006). This ratio is a parameter that may indicate the metabolic pathway favored in the fermentative process. Hydrogen production is linked to organic acid production according to these typical equations based on carbohydrate fermentation (Madigan et al., 2012):

The threshold value of the ratio A/B expressed in units of COD-equivalent is 0.79 if half of biohydrogen comes from Eq. (SM3.1) and the other half comes from Eq. (SM3.2); and the following applies (Robledo-Narvaez et al., 2013):

If (A/B)COD > 0.79; H2 via HAc is likely predominant (SM3.3)

If (A/B)COD < 0.79; H2 via HBu is likely predominant (SM3.4)

For the calculation of the chemical oxygen demand associated to an organic acid we can depart from its oxidation reaction (with O2) as follows

and the COD basis can be determined by:

As an example, for acetic acid:

Therefore, the conversion factor f is:

For butyric acid:

**Appendix 4. Adjustment of carbon-to-nitrogen ratio and initial total solids**

The carbon-nitrogen ratio for the treatments and experimental units of our experiment was adjusted in the range 27 to 43% (Table SM7 below). Given the Nitrogen and organic matter contents of the OFWD and the inoculum as well as the C/N targets of the RSM experimental design, mass balances were performed to estimate the amount of nitrogen to be added to each experimental unit. NH4Cl was selected as the N source. The required amount of N was supplemented as ammonium chloride to each experimental unit.

Ammonium sulfate, although more economic than NH4Cl, was not considered because of the possibility of sulfate-reducing interferences in the anaerobic DF process. Organic sources of N such as peptone, yeast extract, etc. were not considered either because they also add organic carbon thus changing simultaneously the C and the N concentration of the final mixture. Thus, attaining the target C/N in the bioreactors would be more difficult to achieve and likely we should implement additional controls.

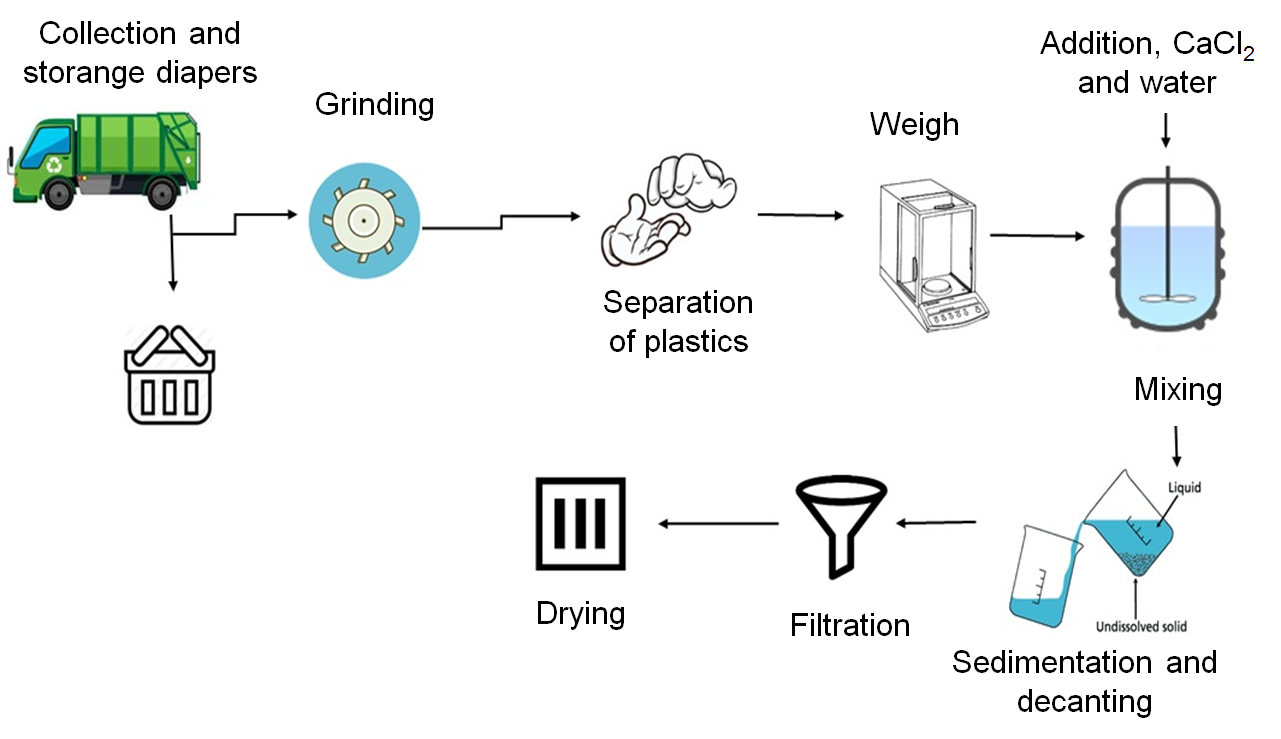
**Table SM7**. Adjustment of Carbon-Nitrogen ratio

|  |  |  |
| --- | --- | --- |
| **C/N (target)** | *OFWD* **(g)** | **NH4Cl (g)** |
| 27 | 0.96 | 0.04 |
| 30 | 0.97 | 0.03 |
| 35 | 0.98 | 0.02 |
| 40 | 0.98 | 0.02 |
| 43 | 0.99 | 0.01 |

The initial total solids concentration was adjusted by addition of a mineral medium. The latter consisted of 31.6 g NaHCO3 and 63.3 g K2HPO4 per liter, pH 7.3.

**Appendix 5. Separation process for the organic fraction of waste diapers**

In our research, only the organic fraction of the diaper (*OFWD*, mainly cellulose) was used, following the protocol described by Girotto et al. (2015). The *OFWD* was prepared as follows (Fig SM1): first, plastics and elastics were separated manually, leaving the core, i.e., SAP, cellulose, feces, and urine. Afterward water was added. CaCl2 was incorporated to reach a target concentration ion of 3,000 mg L-1 in the liquor, to agglomerate and separate the SAP. The mixed liquor was sedimented for 24 h at room temperature. Two phases were formed, the liquid supernatant and the wet solids. The latter, in turn, consisted of 2 cm diameter balls of SAP-Ca and wet *OFWD*. The supernatant was withdrawn by decanting. The SAP-Ca balls were picked-up out from the wet solids phase. The remainder of the wet solids, the *OFWD*, was filtered and further dried at 70 °C for 24 h.



**Fig. SM1.** Separation process for the organic fraction of waste diapers

This procedure has already been carried out by other authors (Torrijos et al., 2013) corroborating its application on an industrial scale, as stated in that article. According to them, in their own words:

“*For the application of the patented process (Conway et al.,1996) to the separation of the constituents of used disposable diapers, the following process, made up of three major successive steps, was set up. (i) First step: coarse shredding to open up the diapers and facilitate subsequent separation of constituents. It should produce plastic pieces large enough in size for them to be removed easily in the second step. Diapers were crushed to a size of approximately 1 cm using a Blik BB 230 crusher equipped with stainless steel rotating blades. (ii) Second step: pulping of the shredded materials and recovery of the plastics. In this step, water was added to reduce dry matter content to a low level (1–2% total solids (TS)) and an adapted mixing was used to permit the ‘‘solubilization’’ of faeces, cellulose and SAP. To prevent the SAP from swelling, a chemical agent was added to the pulping water (dosage used is confidential information). After a few minutes of treatment, the SAP became reticulated and formed small hard particles. At pulper outlet, a screen retained the plastic elements while letting the water containing faeces, cellulose and SAP go through for further treatment. A simple domestic washing machine was used at laboratory scale with a ten-minute washing cycle. Emptying the machine enabled the faeces, cellulose and SAP to be recovered, the plastics remaining in the machine drum. (iii) Third and final step: SAP separation from other components (faeces and cellulose). A simple 100 l tank equipped with a mechanical stiring sys- tem was used. Gentle agitation allowed the cellulose to be maintained in suspension whereas the SAP settled to the bottom of the tank where a draining pipe permitted its recovery. The bio- degradable solid fraction of diapers (BFD), mainly cellulose, was recovered with a 60 lm screen installed along a water recirculation loop on the top part of the 100 L tank. This BFD fraction had a TS concentration of around 10%. Faeces and urine were eliminated with the water flow*”.

**Appendix 6. Analyses of volatile organic acids**

Volatile organic acids also known as volatile organic acids (*VOA*), lactic acid, and solvents were analyzed as reported elsewhere (Muñoz-Páez et al. 2014). An aliquot of the filtrate was injected into a gas chromatography Varian Star 3400 equipped with a flame ionisation detector for metabolite determination. The injector and detector temperatures were set at 250 °C. N2 was used as a carrier gas with a 20 mL min-1 flowrate. The oven temperature was programmed as 60 °C for 2 min, increasing to 140 °C at 5 °C min-1, and then kept constant at 140 °C for another 6 min. A 50 m, 0.32 mm internal diameter fused silica capillary column coated with 0.2 mm CP-Wax 57 CB was used (Sotelo-Navarro et al., 2017).

**Appendix 7. SSAHF-IV process**

The process chosen was based on the ‘solid substrate anaerobic hydrogen fermentation with intermittent venting and headspace flushing’ (SSAHF-IV), that is, when a maximum accumulated concentration of H2 was observed, the headspace of the bioreactors was gassed-out with N2 to expel the accumulated H2. Afterward, the bioreactors were reincubated for another cycle of H2 production and repeated until no more H2 was harvested in the subsequent cycle (Muñoz-Páez et al., 2012; Váldez-Vázquez et al., 2005). Neither new substrate nor fresh inoculum was added during the whole fermentation period (Sotelo-Navarro et al., 2017).

**Appendix 8. Inoculum and seed reactors**

Inocula were drawn from mesophilic, continuous methanogenic solid substrate anaerobic digesters M- SSAD (seed reactors) degrading a mixture of municipal solid wastes operated at 21day mass retention time.

More details regarding the seed reactor start-up, operation and monitoring can be found elsewhere (Poggi-Varaldo et al., 1997). In short, M-SSAD bioreactors were loaded with a 330 g of cattle manure, 330 g of screened garden soil, and 330 mL of waste activated sludge. 10 mL of a stock of concentrated sucrose and sodium bicarbonate was added to each reactor to give a concentration of 2 g sucrose/kg reactor mass con- tent and 4 g CaCO3 alkalinity/kg reactor mass content and manually shaken. Headspace of reactor was flushed with inert N2 gas and subsequently stoppered with a rubber stopper fitted with a nipple, tee, and tubing for biogas exit.

Reactors were connected to acidic brine bio- gas meters and incubated in a mesophilic walk-in room in batch mode for two days. After incipient biogas production, they were fed with 10 mL of sucrose/bicarbonate concentrated stock for a second round of biogas production. This procedure was repeated (usually once more) until at least a concentration of 60% of methane in biogas was reached. Then, reactors were fed twice a week with solid substrate (a mixture of 60% food and 40% paper wastes) at 20% total solids content in such amount to give an average mass retention time of 21 days.

The ratio of substrate-to-inoculum (*OFWD*-to-inoculum) was 10 %. The inoculum was subjected to thermal shock at 92 °C for 30 min that intended to eliminate methanogenic archaea and other H2-consuming microbes from the consortium, as reported elsewhere (Sotelo-Navarro et al. 2017; Muñoz-Paéz et al. 2013).

**Appendix 9. Biohydrogen and organic acids in dark fermentation, which is more sustainable**

On the one side, bioH2 production by dark fermentation of wastes seemed to have gained favor in academic circles (Poggi-Varaldo *et al*., 2014; Ghimire *et al*., 2015; Singh *et al*., 2015). H2 is a very attractive energy carrier and energy source that is claimed to play a significant role in a clean energy era. Yet, the implementation of a sound H2-based economy must overcome several challenges. One of the most significant challenges is H2 storage and distribution (Singh *et al*., 2015). H2 cannot be liquefied at ambient temperature, so, it must be stored either using high pressures at ambient temperature or liquefied at very low temperatures. Both approaches are very expensive and rely on large investment and operation costs, in terms of materials of construction of tanks (both quality and amounts) and energy (either energy for high pressure compression or energy for cryogenic cooling of H2).

Moreover, the infrastructure for mass distribution of H2 is in its infancy. Hydrogen transport/transfer relies on truck transportation; hydrogen duct networks are scarce, if any. Building a H2 distribution infrastructure would imply high investments. Also, the safety and health issues regarding transfer and distribution of H2 should be successfully tackled because there are little full-scale experiences on these issues.

The expenses and technical challenges to be solved can detract from the technical and environmental feasibility of using Hydrogen.

On the other side, VFA and other organic acids (as well as solvents) that can be produced by fermentation of organic wastes are marketable bioproducts; many of them can be used as building blocks in chemical synthesis thus replacing current building blocks from fossil origin, or used for liquid biofuels production, bioelectricity generation or photofermentative bioH2 (Venkata Mohan *et al*., 2016; Ghimire *et al*., 2015)

In another publication that confirmed the importance of organic acids, Venkata Mohan *et al*., (2019) presented a comprehensive panorama focused on the potential applications of waste-derived short chain fatty acids. The authors claimed that these acids are low-cost feedstocks for a great variety of processes that in principle attempt to integrate acidogenic fermentation of wastes with bacteria-based, yeast-based, and photosynthetic-based processes of subsidiary streams.

The sub family of C1–C7 acids are referred to as “volatile fatty acids” (VFAs) because of their relatively high vapor pressure and high volatility, when compared to other carboxylic acids. In addition to C1-C7, other organic acids as well as solvents could be of interest in this discussion. A short list of organic acids of interest include value-added as well as commodities compounds, such as succinic acid, lactic acid, pyruvic, malic, fumaric, 2,5-furan dicarboxylic acid, 3-hydroxy propionic acid, as­partic acid, glucaric acid, glutamic acid, itaconic, levulinic, citric, and C1 to C7 short-chain fatty acids (Werpy & Petersen, 2004; Brown, 2007; Murali *et al*., 2017). Other researchers have mentioned organic compounds other than acids, such as the polyols glycerol, sorbitol, and xylitol/arabinitol, one ketone (3-hydroxybutyrolactone) and 1, 3-propanediol as attractive building block molecules (Cameron *et al*., 1998; Kurian, 2005), as well as conventional low molecular weight solvents (ethanol, acetone, butanol).

In the recent past, most carboxylic acids used by the food, pharmaceutical, chemical, and fuel industries were produced from fossil fuels. Efforts have been made to produce organic acids from waste and biomass using biochemical processes. A successful case of biotechnological production of an organic acid is the lactic acid production from corn sugars (Murali *et al.,* 2017). Years ago, chemical synthesis of lactic acid started by performing an acid hydrolysis of lactonitrile (Holten *et al*., 1971; John *et al*., 2007).

Afterward, sugars were catalytically degraded to propylene glycol and other compounds. Further oxidation of the latter plus catalytic reactions of acetaldehyde, CO, and H2O lead to lactic acid. Murali *et al*., (2017) pointed out that this process had several disadvantages*, inter alia*, large energy consumption, high costs (both investment and operating), and the final product was a mixture of D- and L- lactic acids. Now, biotechnological production of commercial lactic acid has completely replaced its production based on chemical synthesis. According to a commercial Internet report, by 2020, the world lactic acid market would reach USD 3.82 billion by 2020, with a growth rate of ca. 19% the forecast period. In 2015, the market is estimated to be dominated by North America, followed by Asia-Pacific. The world polylactic acid market would be around USD 5.16 billion with a growth rate of ca. 21%. A few years ago (2015) the market was dominated by Europe, followed by North America [Lactic acid market by application (Biodegradable Polymer, Food & Beverage, Personal Care & Pharmaceutical) & polylactic acid market by application (Packaging, Agriculture, Automobile, Electronics, Textile), & by Geography-Global Trends & Forecasts to 2020. 2015. Available online: http://www.rnrmarketresearch.com/lactic-acid-market-by-application-biodegradable-polymer-food-beverage-personal-care-pharmaceutical-polylactic-acid-market-by-application-packaging-agriculture-automobile-electronics-tex-market-report.html (Accessed on August 30, 2019).

Therefore, there is a strong market out there for organic acids from dark fermentation. In fact, organic acids can be considered an attractive platform for a great variety of profitable applications, such as “bio(petro)chemistry”, gas and liquid biofuels, pharmaceuticals, and more. Another advantage is that organic acids and further storage and distribution are technically solved, since it is a part of the current industrial practice, although separation processes can account for over 30% or more of the total costs of processing carboxylic acids (Murali *et al*., 2017). Typical unit operations applied for In situ or on-line product recovery of carboxylic acids from their fermentation broths include distillation, dialysis, extraction, and adsorption (Freeman *et al*., 1993). In particular, extraction and adsorption have been applied often in the continuous acid recovery from anaerobic fermentation.

Yet, please note that bioH2 and organic acids are typically co-produced in dark fermentation when using organic wastes as substrate and undefined anaerobic microbial consortia (Ghimire *et al.*, 2015; Valdez-Vazquez and Poggi-Varaldo, 2009). In a context of favoring the production of organic acids over the production of bioH2, it seems that there is a need of certain process strategies.

For instance, fostering processes and microbes that could affect the anaerobic degradation of the wastes by other metabolic routes with less or no bioH2 production allowing for the accumulation of organic acids and other organic compounds (Nath and Das, 2004; Hallenbeck and Benemann, 2002). As an example of the first type of pathway it is well known that some clostridia favor the generation of 2 moles of H2 and butyrate from hexose fermentation, whereas *Clostridium pasterianum* fermentation of hexoses leads to the formation of 4 moles of H2 (Eq. SM9.1 and SM9.2, respectively).

C6H12O6 → CH3CH2CH2COOH + 2H2 + CO2 [SM 9.1]

C6H12O6 + 4H2O → 2CH3COOH + 4H2 + 2HCO3- + 2H+ [SM 9.2]

Another case of the first category consists of metabolic pathways that lead to ethanol and acetate production lowering the stoichiometric yield of H2 to 2 moles of H2/mole of hexose as discussed by Li and Fang (2007) (Eq. SM9.3)

C6H12O6 + 3H2O → CH3CH2OH + CH3COOH + 2H2 + 2HCO3- + 2 H+ [SM9.3]

A strategy of the second type could consist of choosing special fermentation processes/microorganism that do not produce H2 or, better, are a sink of H2. Lactic fermentation is known to produce two moles of lactic acid from hexoses (homolactic fermentation) and no bioH2 at all (Escamilla-Alvarado *et al*., 2012). Fermentation leading to low molecular weight organic solvents is also recognized to be a “sink” of hydrogen (Ghimire *et al*., 2015).

Another example could be the mixed fermentation of hexoses by *Clostridium barkeri* that produce only lactic acid plus ethanol (represented by Eqs. SM9.4 and SM9.5) below:

C6H12O6 + 2H2O → CH3CHOHCOOH + 2HCO3- + 2H+ [SM9.4]

C6H12O6 + 2H2O → 2 CH3CH2OH + 2HCO3- + 2H+ [SM9.5]

Also, *Clostridium articum*, which can perform as a H2-consuming microbe, gives another example of a “H2-sink” strategy (Kim *et al*., 2006) that allows for the accumulation of propionic acid not only without accumulation of H2, but rather the consumption of H2, Eq. SM9.6.

C6H12O6 + 2H2 → 2CH3CH2COOH + 2H2O [SM9.6]

Also, metabolic engineering and genetic modifications were reported to lead to organic acid yield improvements, particularly butyric acid (Xu *et al*., 2014; Liu *et al*., 2006; Zhang *et al*., 2009; Cameron *et al*., 1998).

Sometimes, a strategy as simple as changing the pH in the fermentation could be a useful tool to increase organic acids yields: Liu *et al*., (2006) achieved the highest butyrate production by *Clostridium tyrobutyricum* at a pH between 6.0-6.7. The authors suggested that the shift in metabolic flux was a function of pH and it was related to the difference in activities of phosphortransbutyrylase, phosphotransacetylase, and lactate dehydrogenase.

However, the above-mentioned strategies for improving yields of organic acids (and possibly solvents) as products of dark fermentation imply some technical challenges and additional costs, such as energy expenses for substrate sterilization, investment and operating costs for special inocula culturing, supplementing chemicals, etc. As a general remark, any strategy for the above-mentioned purpose that needs to resort to a pure culture would have the burden of energy consumption for sterilization which is an obvious disadvantage.

Finally, the issue on which could be more sustainable, i.e., H2 or organic acids from waste fermentation, should be decided according to:

*(i)* a market analysis that identifies the types of organic acids of interest in the region and warrants their use,

*(ii)* a careful analysis that applies System Analysis tools that would complete the feasibility study. For example, life cycle assessment and life cycle costing would shed light in a more objective way on the advantages of one approach (biohydrogen) or the other (organic acids) from dark fermentation, in terms of their impacts to the environment, health, and resources, as well as the economic impacts (Venkata Mohan *et al.*, 2016; Ahlgren *et al*., 2013; Ahlgren *et al*., 2015; Escamilla-Alvarado *et al*., 2017).

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