Electronic Supplementary Information

Synergetic conversion of microalgae and CO_2 into value-added chemicals under hydrothermal conditions

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Experimental Section

Materials

In this study, *Spirulina* and *Chlorella* were selected as the representative of microalgae and were purchased from Yunnan Natural Biotech Co., Ltd. and Shandong Binzhou TianJian Biotechnology Co., Ltd., respectively. The solid residue of *Chlorella* after biofuel extraction was also used. The detailed chemical composition of *Spirulina* is shown in Table S1, S2 and S3†, and the ultimate analysis of *Spirulina*, *Chlorella* and *Chlorella* residue can be found in Table S5†. Sodium bicarbonate ($NaHCO_3$, 99.5%, Sinopharm Chemical Reagent Co., Ltd) was used as the CO_2 source for considerations that $NaHCO_3$ is the product of trapping CO_2 from waste streams by basic solution and also experiental convenience. Bovine serum albumin (BSA, N>13.5%). 2-pyrrolidinone (99%), 2-piperidinone (99%), acetamide were used for mechanism study and were purchased from Sinopharm Chemical Reagent Co., Ltd, Alfa Aesar., Sigma-Aldrich., and J&K Scientific Ltd., respectively. $NaH^{13}CO_3$ (CP, ≥97%), $H^{13}COOH$ and $CH_3{}^{13}COOH$ (≥95%) were used to determine the carbon source of formate and were bought from Cambridge Isotope Laboratories, Inc. All reagents were used without further purification. Deionized water was used throughout the study.

Experimental procedures

Experiments were conducted with a series of stainless steel (SUS 316) tubular reactors with two end fittings, giving an inner volume of 5.7 mL. The schematic drawing of the experimental set-up can be found in our previous report.[1] A typical reaction procedure is as follow. The desired amount of $NaHCO_3$, *Spirulina* (0.28 g), and deionized water were loaded into the reactor. Then the reactor was sealed and put into a salt bath that was preheated to the desired temperature. At a selected reaction time, the reactor was removed from the salt bath and immediately

immersed into a cold-water bath to quench the reaction. When BSA (or 2-pyrrolidinone, 2-piperidinone) was used instead of the *Spirulina* in the reaction for mechanism study, or *Chlorella* and its residue were used to expand the microalgae source, their quantities were determined based on the N molar amount, which were the same as that contained in 0.28 g *Spirulina*, namely 2.1 mmol.

Definitions

The reaction time was defined as the duration of the reactor being kept in the salt bath. The water filling was defined as the volume of the solution in the reactor divided by the total inner volume of the reactor. The formate yield was used to evaluate the efficiency of $NaHCO_3$ reduction by microalgae, and was calculated as follow:

$$Formate\ Yield = \frac{Formate\ from\ NaHCO_3\ (mmol/L)}{The\ intial\ NaHCO_3\ (mmol/L)}$$

Analytical methods

All liquid samples were collected, filtered through a 0.22 μm syringe filter and then analyzed by high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), gas chromatography-flame ionization detector (GC-FID) and ^{13}C-quantitative nuclear magnetic resonance (^{13}C-QNMR). Gaseous samples were analyzed by gas chromatography-thermal conductivity detector (GC-TCD).

1) High-performance liquid chromatography (HPLC)

The HPLC analysis was performed on an Agilent 1200 system, which was equipped with two KC-811 columns (SHODEX) for sample separation and a tunable UV/Vis absorbance detector adjusted at 210 nm for sample detection. 2 mmoL/L $HClO_4$ solution at a flow rate of 1.0 mL/min was used as the mobile phase. The concentration of formate, acetate and propionate were

quantified by HPLC, and were obtained using the external standard method based on the average values of at least three parallel experiments.

2) Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis was used for product identification. A Hewlett-Packard model 7890A GC system equipped with a model 5975C mass selective detector (MSD) was used. Separation of samples was achieved with an Agilent 19091N-233HP-INNOWax Polyethylene Glyco column (30 m x 250 μm x 0.5 μm) with He as carrier gas at a flow rate of 1 mL/min. The oven temperature was initially held at 40 ºC for 4 min, and then procedurally increased at a rate of 7 ºC/min to a final temperature of 230 ºC and was held for 20 min.

3) Gas chromatography-flame ionization detector (GC-FID)

The concentration of N-methyl-2-pyrrolidinone, 2-pyrrolidinone and 2-piperidinone were quantified by GC-FID, using the external standard method based on the average values of at least three parallel experiments. A Hewlett-Packard model 5890 Series II GC system equipped with a flame ionization detector (FID) was used. The samples separation was achieved with an Agilent 19091N-233HP-INNOWax Polyethylene Glyco column (30 m x 250 μm x 0.5 μm), and He at a flow rate of 1 mL/min was used as the carrier gas. The program procedures for oven operation were the same to GC-MS analysis.

4) ^{13}C-quantitative nuclear magnetic resonance (^{13}C-QNMR)

^{13}C-QNMR analysis was used to determine the concentration of formate from $NaHCO_3$. Before analysis, liquid sample was sealed with 100 mmol/L $CH_3{}^{13}COOH$ as the internal standard in a NMR tube (5 mm i.d.). ^{13}C-QNMR analysis was then performed on a Bruker Avance Ⅲ 600 MHz NMR-Spectrometer.

The calculation of $H^{13}COOH$ concentration was based on the following formula:

$y=0.5414x+0.135$, $R^2=0.9975$

where x represents $H^{13}COOH$ concentration/$CH_3{}^{13}COOH$ concentration, and y represents $H^{13}COOH$ area/ $CH_3{}^{13}COOH$ area in ^{13}C-QNMR spectra.

The formula was obtained by examining the ratio of $H^{13}COOH$ to $CH_3{}^{13}COOH$ based on the peak area in the ^{13}C-QNMR spectra when varying the concentration of $H^{13}COOH$ and $CH_3{}^{13}COOH$. The peak area ratios with different concentrations of $H^{13}COOH$ and $CH_3{}^{13}COOH$ are summarized in Table S6†.

5) Gas chromatography-thermal conductivity detector (GC-TCD)

GC-TCD analysis was used to determine the gaseous reducing products from CO_2. A HP-5890 Series II with an HP-1 packing column was used.

Tables and figures

Table S1 Basic chemical composition of *Spirulina*

Chemical composition	Protein	Carbohydrate	Lipid	Water	Ash
Relative amount in *Spirulina* (%)	70	12	9	6	3

Table S2 Amino acids compositions of the protein contained in *Spirulina*[2]

Protein	composition (%)
Alanine	7.2
Arginine	6.7
Aspartic acid	8.7
Glutamic acid	11.1
Glycine	5
Histidine	2.0
Isoleucine	6.3
Leucine	8.3
Lysine	4.2
Methionine	0.9
Phenylalanine	5.4
Proline	4.0
Serine	4.0
Threonine	4.4
Tyrosine	4.1
Valine	6.7
Other	11

Table S3 Lipids profile of the tested *Spirulina*[3]

	Lipid	Composition (%)
Non-polar lipid (9%)	Pigments and polar lipids	0.86
	Monoglycerides	0.74
	Free sterols	0.14
	Diglycerides	0.32
	Free fatty acids	6.2
	Triglycerides	0.74
Polar lipid (91%)	Sterol esters, waxes, etc.	3.9
	Digalactosyl diglyceride	22.5
	Phosphatidyl glycerol	23.6
	Unresolved lipid	41

Main component fatty acid is palmitic acid.

Table S4 Major products for the *Spirulina*/BSA/glucose/palmitic acid reaction with or without $NaHCO_3$[a]

		Products concentration (mmol/L)					
		Formate	Acetate	Propionate	N-methyl-2-pyrrolidinone	2-pyrrolidinone	2-piperidinone
Spirulina	With $NaHCO_3$	114.5[b]	76.4	37.5	16.3	4.2	6.6
	Without $NaHCO_3$	5.3	19.2	3.4	7.5	40.9	35.6
BSA	With $NaHCO_3$	106.6[b]	40.4	40.5	15.6	7.6	8.7
	Without $NaHCO_3$	3.5	16.2	3.2	6.8	42.7	36.4
Glucose	With $NaHCO_3$	7.3[b]	25.8	-	-	-	-
	Without $NaHCO_3$	2.3	2.7	-	-	-	-
Palmitic acid	With $NaHCO_3$	-	-	-	-	-	-
	Without $NaHCO_3$	-	-	-	-	-	-

[a]Reaction conditions: 1.2 mol/L $NaHCO_3$, 0.28 g *Spirulina*, 0.75 mol/L BSA, 0.13 mol/L glucose, 0.09 mol/L palmitic acid, 300 °C, 2 h, 50% water filling.
[b]Total formate concentration, which consists of formate from $NaHCO_3$ reduction and *Spirulina* model compounds conversion.

Table S5 Ultimate analysis of *Spirulina, Chlorella* and *Chlorella* residue[a]

	C (wt %)	O (wt %)	N (wt %)	H (wt %)	Else (Ca S P) (wt %)
Spirulina	48.2	34.3	10.7	6.8	0.6
Chlorella	45	39.84	8.26	6.8	0.1
Chlorella residue	40.2	43.84	8.4	6.3	1.26

[a]Results were obtained with the elemental analyzer (Vario EL III).

Table S6 ^{13}C-QNMR analysis of H^{13}COOH and CH$_3$^{13}COOH

Entry	H^{13}COOH concentration (mmol/L)	CH$_3$^{13}COOH concentration (mmol/L)	H^{13}COOH concentration/ CH$_3$^{13}COOH concentration	H^{13}COOH area/ CH$_3$^{13}COOH area
1	50	100	0.5	0.39
2	100	100	1.0	0.70
3	100	50	2.0	1.21

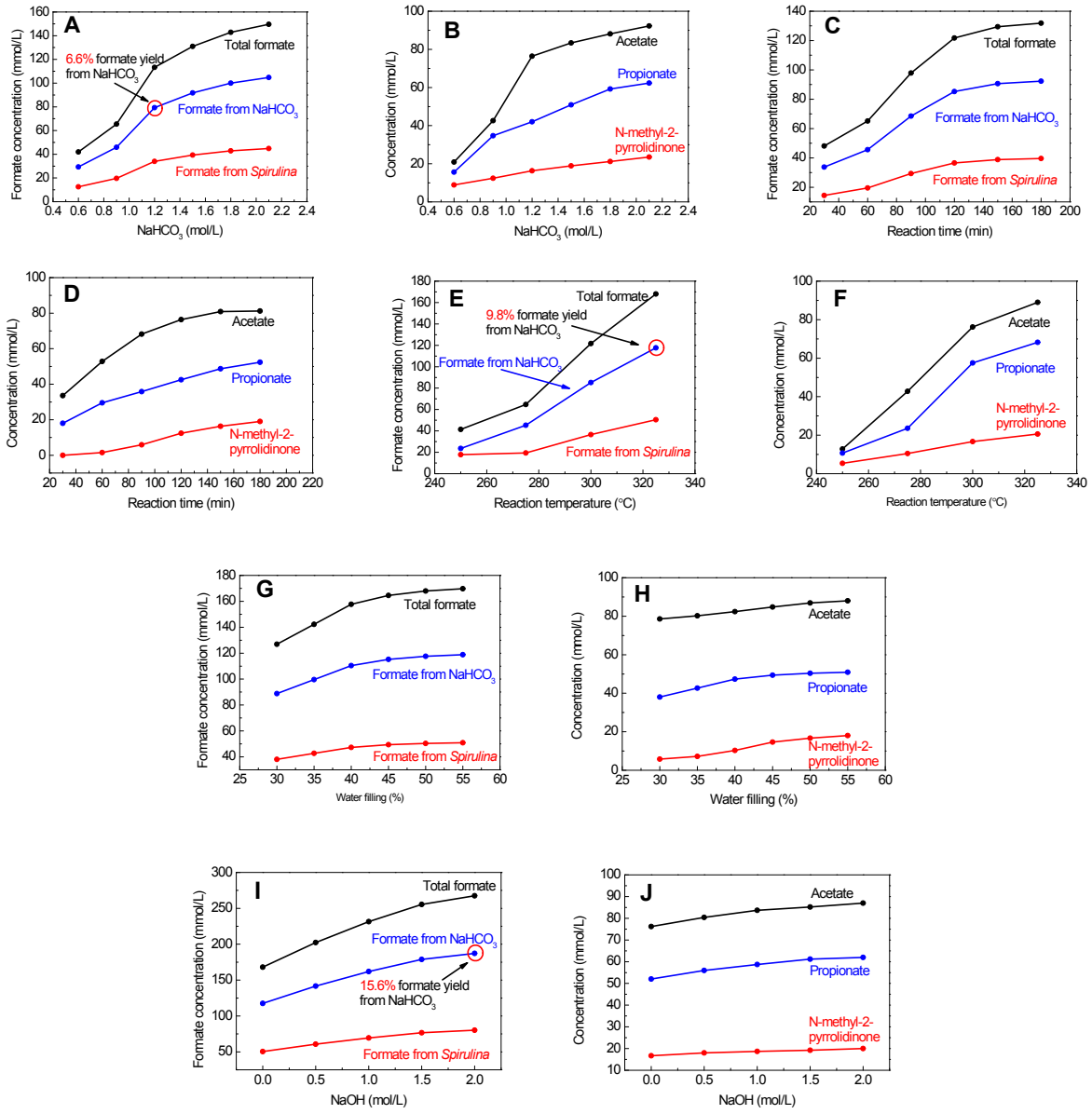

Fig. S1 Effect of initial $NaHCO_3$ concentration (A and B), reaction time (C and D), temperature (E and F), water filling (G and H) and NaOH concentration (I and J) on the conversion of *Spirulina* and $NaHCO_3$ (Reaction condition: (A and B) 0.28 g *Spirulina*, 300 °C, 2 h, 50% water filling; (C and D) 1.2 mol/L $NaHCO_3$, 0.28 g *Spirulina*, 300 °C, 50% water filling; (E and F) 1.2 mol/L $NaHCO_3$, 0.28 g *Spirulina*, 2 h, 50% water filling; (G and H) 1.2 mol/L $NaHCO_3$, 0.28 g *Spirulina*, 325 °C, 2 h; (I and J) 1.2 mol/L $NaHCO_3$, 0.28 g *Spirulina*, 325 °C, 2 h, 50% water filling).

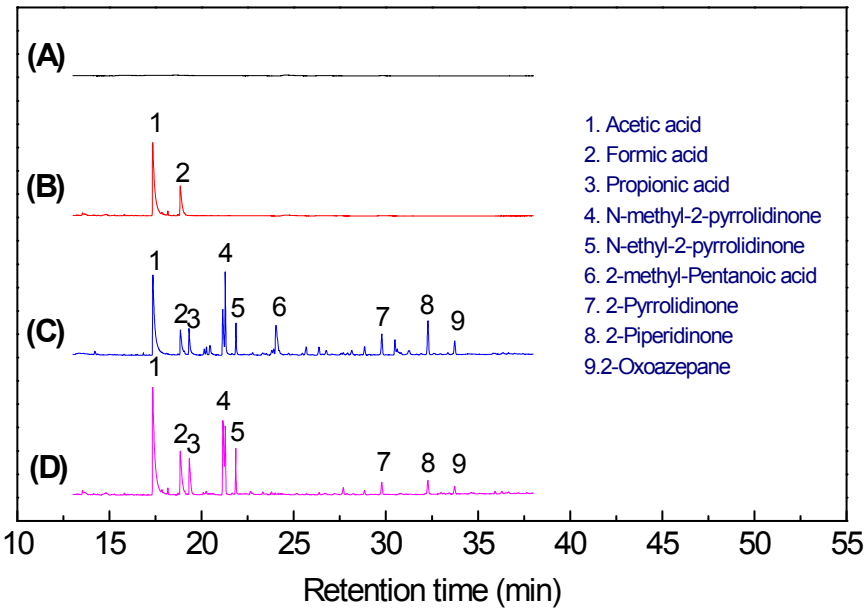

Fig. S2 GC-MS chromatograms of liquid samples for the reaction of $NaHCO_3$ with palmitic acid (A), glucose (B), bovine serum albumin (BSA) (C) or *Spirulina* (D) (1.2 mol/L $NaHCO_3$, 0.09 mol/L palmitic acid, 0.13 mol/L glucose, 0.75 mol/L BSA, 0.28 g *Spirulina*, 2 h, 300 ºC, 50% water filling).

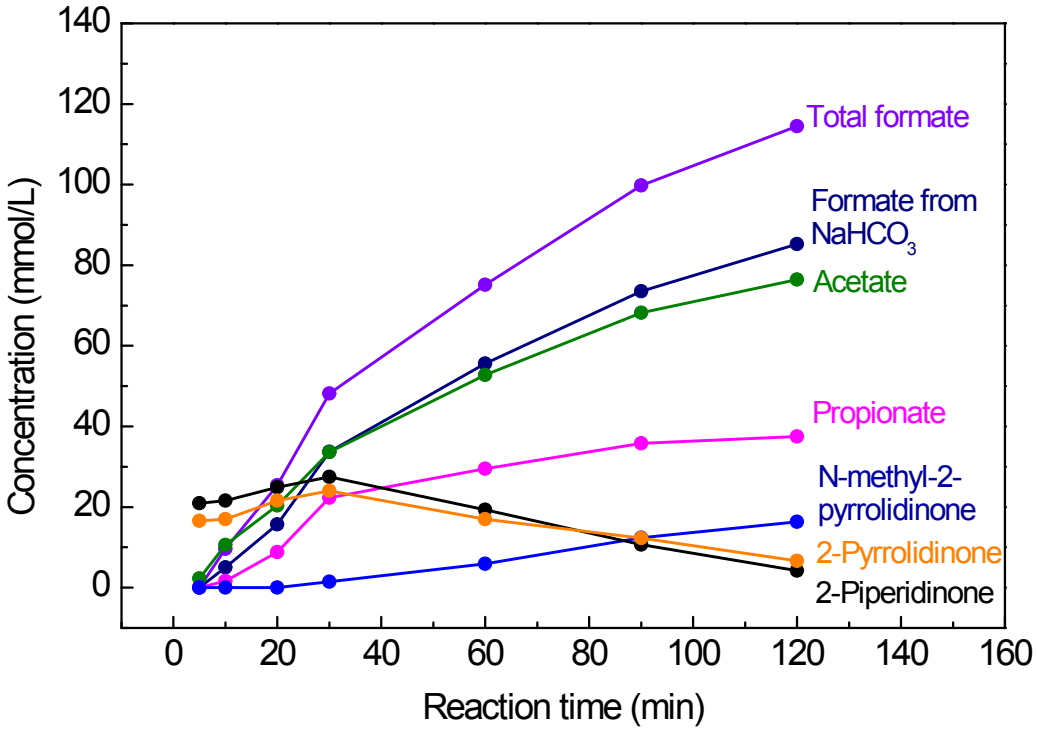

Fig. S3 Product concentration as function of time obtained in the reaction of *Spirulina* with $NaHCO_3$ (1.2 mol/L $NaHCO_3$, 0.28 g *Spirulina*, 300 ºC, 50% water filling).

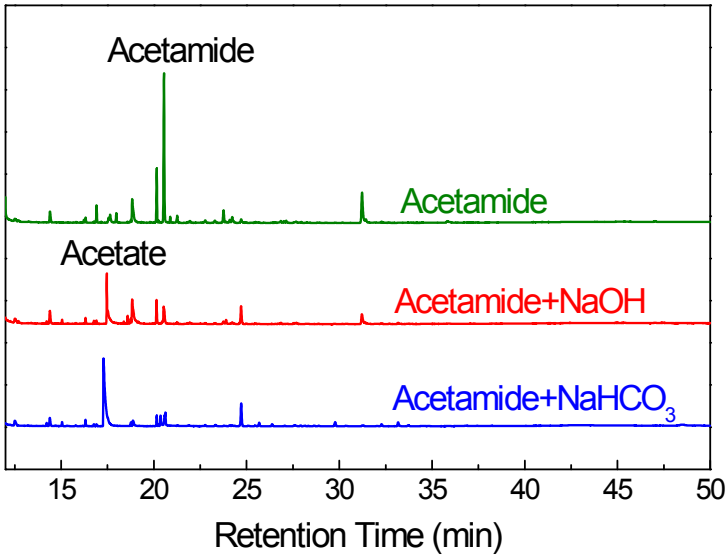

Fig. S4 GC-MS chromatograms of liquid samples for the reaction of acetamide with NaOH or $NaHCO_3$ (1 mol/L acetamide, 1 mol/L $NaHCO_3$, 1 mol/L NaOH, 2 h, 300 ºC, 50% water filling).

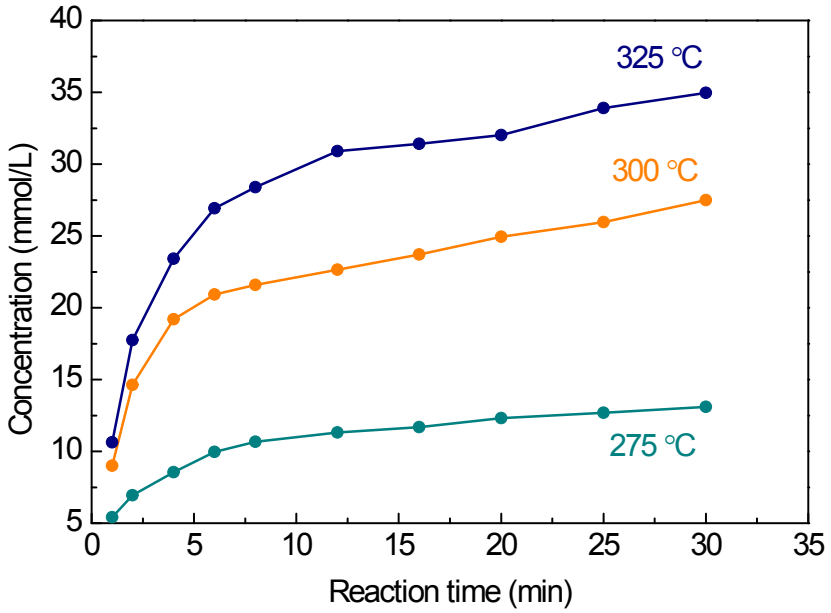

Fig. S5 Kinetic information of lactam (2-pyrrolidinone) formation from the reaction of *Spirulina* with $NaHCO_3$ (1.2 mol/L $NaHCO_3$, 0.28 g *Spirulina*, 300 ºC, 50% water filling).

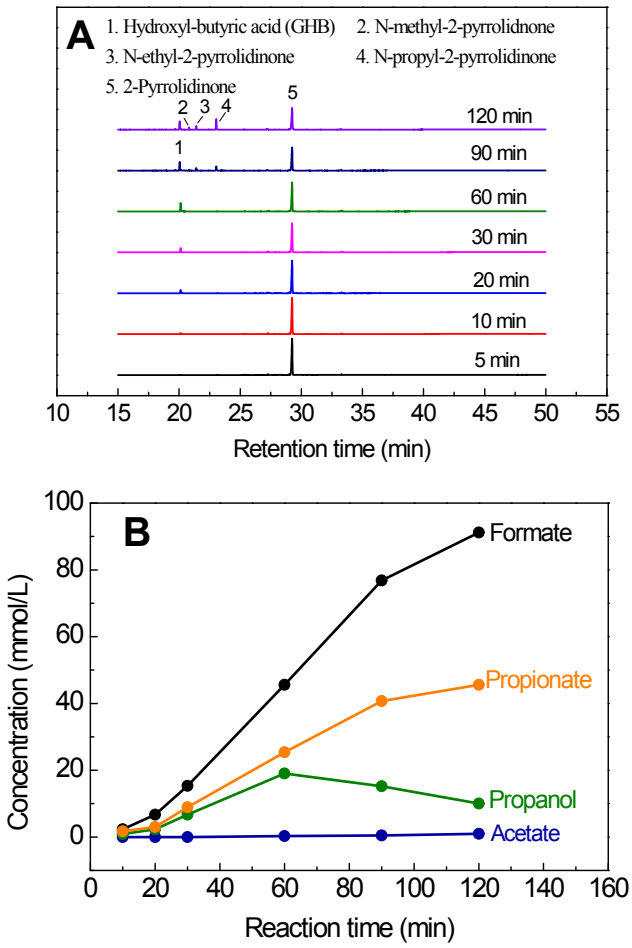

Fig. S6 GC-MS chromatograms (A) and products variation with time (B) from the reaction of 2-pyrrolidinone with $NaHCO_3$ (Reaction conditions: 1.2 mol/L $NaHCO_3$, 0.75 mol/L 2-pyrrolidione, 300 ºC, 50% water filling).

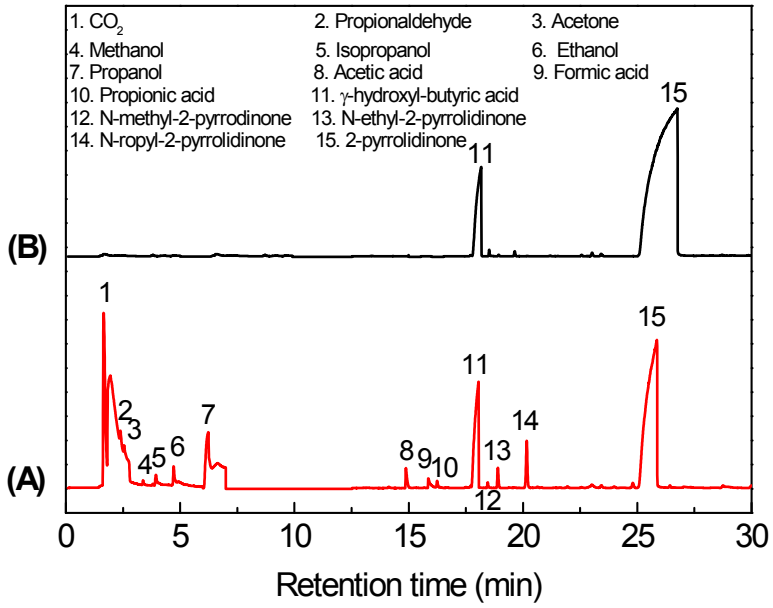

Fig. S7 GC-MS chromatograms of liquid samples after the reaction of 2-pyrrolidinone with (A) or without $NaHCO_3$ (B) (1.2 mol/L $NaHCO_3$, 0.75 mol/L 2-pyrrolidinone, 2 h, 300 ºC, 50% water filling).

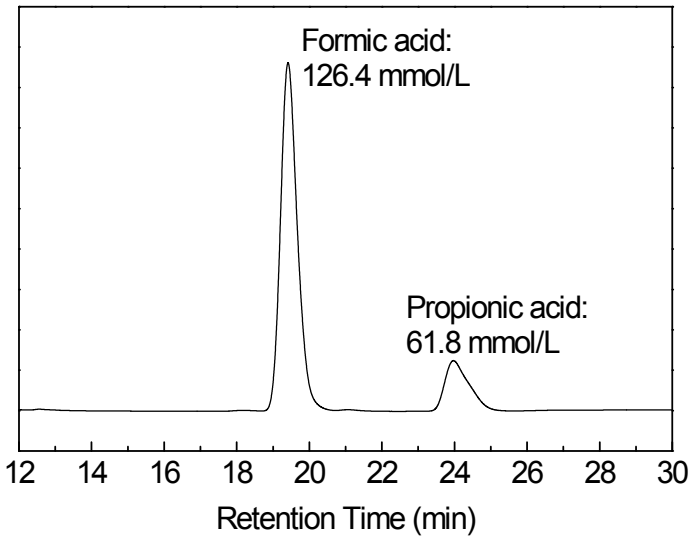

Fig. S8 HPLC chromatogram after the reaction of propanol with $NaHCO_3$ (1 mol/L $NaHCO_3$, 1 mol/L propanol, 2 h, 300 ºC, 50% water filling).

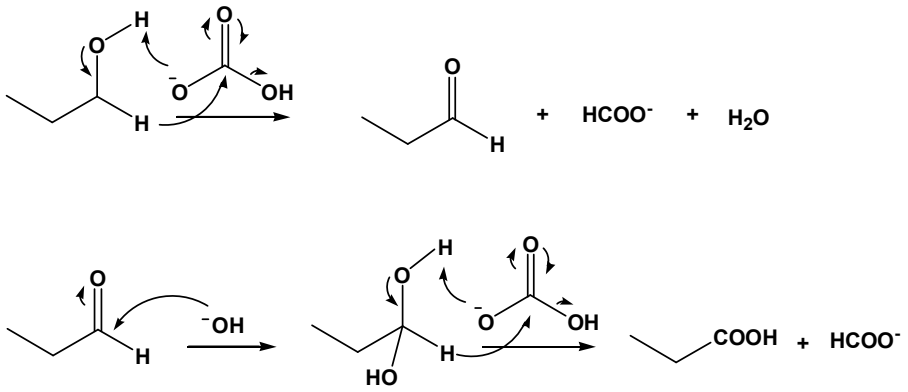

Fig. S9 Proposed mechanism for $NaHCO_3$ reduction with propanol.

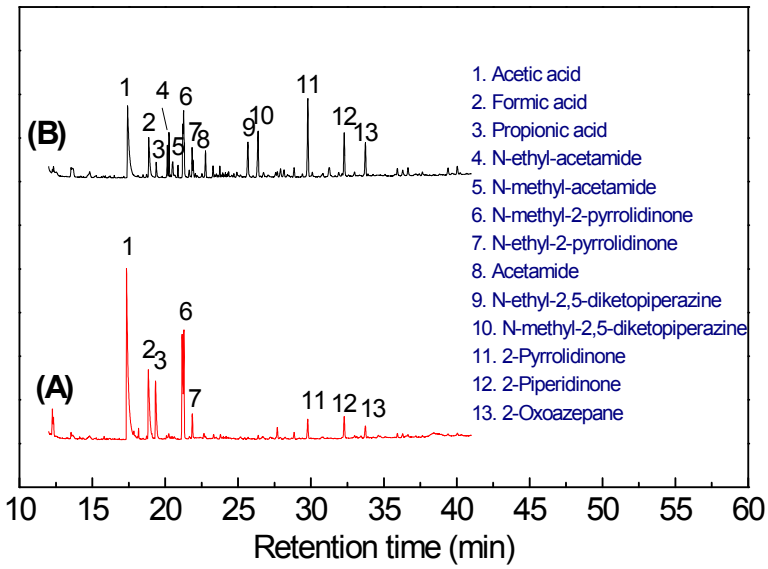

Fig. S10 GC-MS chromatograms of liquid samples after the reaction of *Chlorella* with (A) or without $NaHCO_3$ (B) (Reaction condition: 1.2 mol/L $NaHCO_3$, 0.36 g *Chlorella*, 2 h, 300 ºC, 50% water filling).

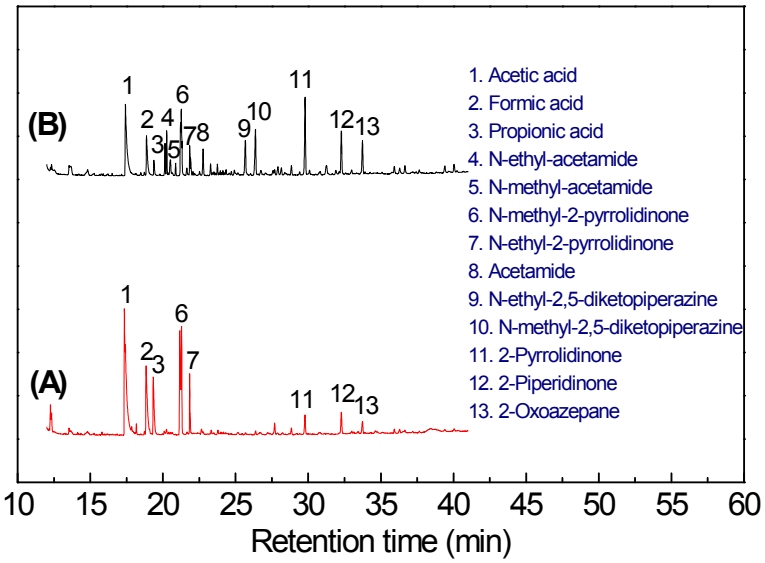

Fig. S11 GC-MS chromatograms of liquid samples after the reaction of *Chlorella* residue with (A) or without $NaHCO_3$ (B) (Reaction condition: 1.2 mol/L $NaHCO_3$, 0.35 g *Chlorella* residue, 2 h, 300 ºC, 50% water filling).

References:

1. F. Jin, A. Kishita, T. Moriya and H. Enomoto, *J. Supercrit. Fluids*, 2001, **19**, 251-262.

2. C. I. Waslien and W. Oswald, Crit. *Rev. Food Sci. & Nutri.*, 1975, **6**, 77-151.

3. B. J. Hudson and I. G. Karis, *J. Sci. Food. Agric.*, 1974, **25**, 759-763.