# Sustainable separation of bio-based cadaverine based on carbon dioxide capture by forming carbamate

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## Methods

### Materials and chemicals

Cadaverine bioconversion fluid was obtained from our laboratory and referred to Ma′s methodology [1]. Cadaverine standard (> 99.6%) was prepared by our laboratory. Pure cadaverine carbamate (> 99.5%) was prepared by the direct injection of carbon dioxide into pure cadaverine (> 99.6%) and then precipitation was dried to a constant weight in our laboratory. Carbon dioxide, derived from the decarboxylation of L-lysine, was collected and stored in pressure storage tanks. Granular activated carbon (JK1 and JK2) and powder activated carbon (SF1 and SF2) were purchased from Sinopharm Chemical Reagent Co., Ltd (China). N-butanol was purchased from Aladdin (China).

### Decolorization of concentrated cadaverine solution by activated carbon

Here, activated carbon was used to remove the pigment from concentrated cadaverine solution. In a 250 mL flask containing 50 mL of concentrated cadaverine solution, effects of types of activated carbon including granular activated carbon (JK1 and JK2) and powder activated carbon (SF1 and SF2) on decolorization efficiency and cadaverine yield were studied. Then, effects of the content of activated carbon (0.5, 1.0, 1.5, 2.0, and 2.5%), decolorization speed (100, 150, 200, 250, and 300 rpm), decolorization time (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 h), and decolorization temperature (18, 25, 30, 33, and 37 ℃) on decolorization efficiency and cadaverine yield were investigated. After decolorization, samples were centrifuged at 12000 rpm for 5 min at room

temperature. The supernatant was diluted with for 25 times by distilled water, and the absorbance value was measured at 405 nm by an UV-VIS spectrophotometer.

Decolorization efficiency (%) = $(A_0-A_1)/A_0$ *100%

where $A_0$ is the absorbance value before decolorization; $A_1$ is the absorbance value after decolorization.

**Effect of cadaverine concentration in the deprotonation cadaverine extraction solution on the yield and purity of carbamate**

Influences of different concentrations of cadaverine (60.4, 134.2, and 272.0 g $L^{-1}$) in n-butanol extraction solution at 250 mL flask containing 50 mL of cadaverine extraction solution on the yield and purity of cadaverine carbamate were studied at a constant flow rate of carbon dioxide with 5 mL $min^{-1}$ for 30 min.

**Refined cadaverine carbamate by solventing-out crystallization**

The crude cadaverine carbamate was used as raw material, ethanol as solventing-out solvent, and crude cadaverine carbamate solution as main solvent, and then the yield and purity of cadaverine carbamate were investigated after solventing-out crystallization. Under the conditions of 50 mL at concentration of 600 g $L^{-1}$ of crude cadaverine carbamate, crystallization temperature at 5 ℃, solventing-out solvent flow rate at 0.11 mL $min^{-1}$, volume ratio of solventing-out solvent to main solvent at 1:6, and stirring speed at 200 rpm, the yield and purity of refined cadaverine carbamate were studied by solventing-out crystallization with ethanol for three times.

**Determination of purity and yield of cadaverine carbamate**

Samples of crude or refined cadaverine carbamate and equal mass pure cadaverine carbamate were dissolved in an aqueous solution. Cadaverine carbamate was analyzed by Agilent 1260 HPLC (USA) with an Agilent YMC Carotenoid column (250 mm×4.6 mm, 5 μm) equipped with an Agilgent 1260 infinity differential refractometer detector. An amount of 10 μL samples was eluted at a rate of 0.8 mL/min by a mixture of 0.1% trifluoroacetic acid (TFA) in 100% water and 5% acetonitrile in 100% water with the column temperature of 35℃.

The purity cadaverine carbamate was calculated based on the pure cadaverine carbamate made in our laboratory.

Purity of cadaverine carbamate (%) = Peak area of sample of crude or refined cadaverine carbamate / Peak area of equal mass pure cadaverine carbamate

Cadaverine carbamate was dissolved in aqueous solution to release cadaverine, the concentration of cadaverine in aqueous solution was determined by the HPLC.

Yield of cadaverine carbamate (%) = Total cadaverine in the cadaverine carbamate / Total cadaverine in the butanol extract or solution

**Characterization of cadaverine carbamate**

Element (C, N, O, and H) contents of refined cadaverine carbamate were analyzed by an elementar vario MICRO cube organic element analyzer (Germany). Fourier transforms infrared spectroscopy (FTIR) of crude or refined cadaverine carbamate was

analyzed by a Thermo Corporation Nexus spectrophotometer (USA) within a range of 500–4000 $cm^{-1}$. Thermogravimetric analysis (TGA) of refined cadaverine carbamate was performed using a TA Q500 (USA) at a heating rate of 10 °C/min in a temperature range of 50–400 °C. X-ray di□raction (XRD) of refined cadaverine carbamate was performed on a Rigaku Ultima IV di□ractometer with Cu Kα X-rays. GC-MS of refined cadaverine carbamate and free cadaverine was performed using a Thermo Scientific ISQ 7000 mass spectrometer with EI mode. $^{13}C$ and $^{1}H$ nuclear magnetic resonance (NMR) spectroscopy of refined cadaverine carbamate and free cadaverine was performed using a Bruker AVANCE III HD NMR spectrometer.

## References

1 W. Ma, W. Cao, H. Zhang, K. Chen, Y. Li and P. Ouyang, *Biotechnol. Lett.,* 2015, **37**, 799–806.

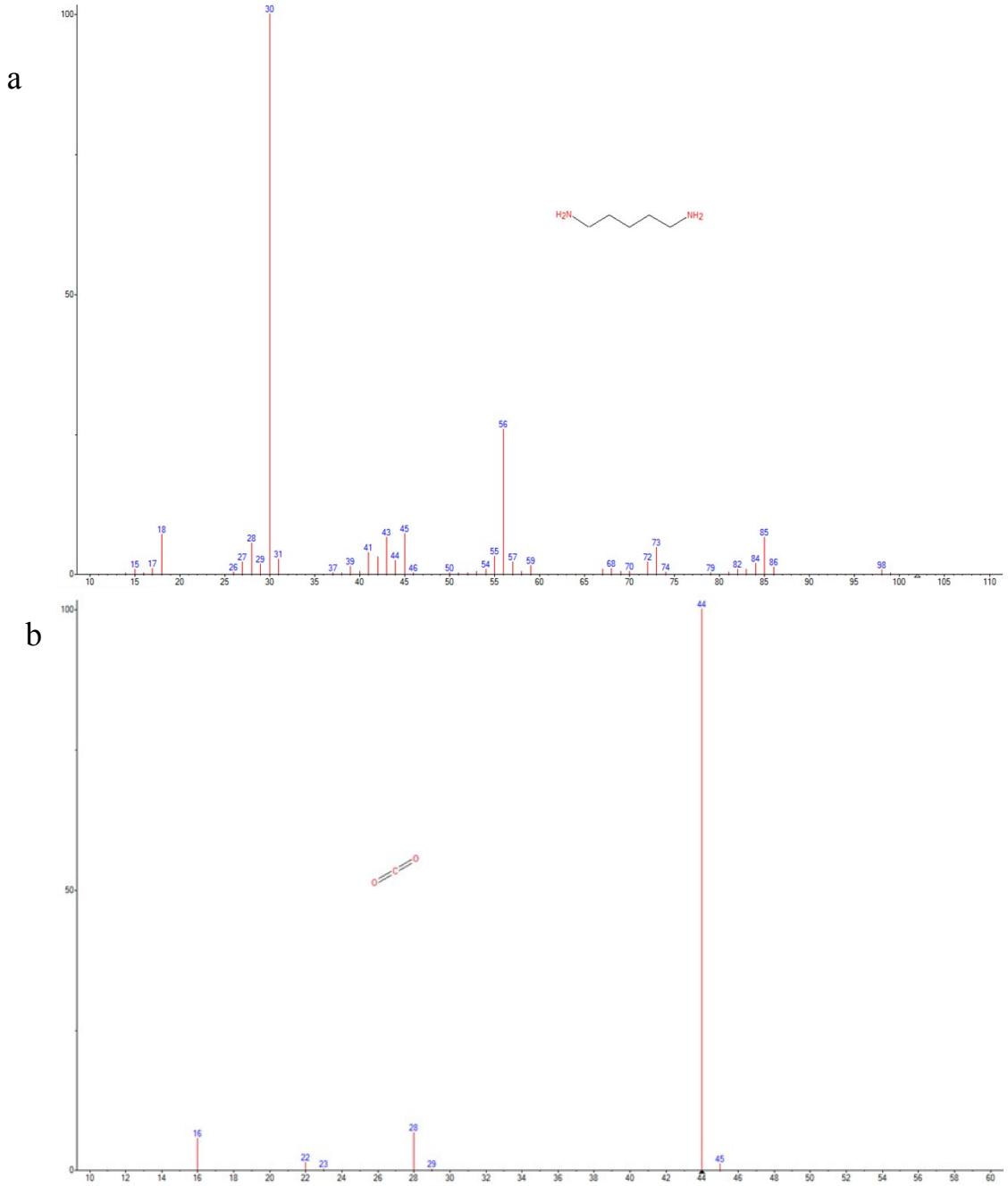


**Fig.S1** EI-MS of cadaverine (a) and $CO_2$ (b) from refined cadaverine carbamate and free cadaverine