Toward nitrogen-neutral bio-energy from renewable protein waste and CO_2

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The non-recyclable use of nitrogen fertilizers in microbial production of fuels and chemicals remains environmentally detrimental. Conversion of protein wastes into biofuels and ammonia by engineering nitrogen flux in Escherichia coli has been demonstrated as a method to reclaim reduced-nitrogen and curb its environmental deposition. However, protein biomass requires a proteolysis process before it can be taken up and converted by any microbe. Here, we metabolically engineered Bacillus subtilis to hydrolyze polypeptides through its secreted proteases and to convert amino acids into advanced biofuels and ammonia fertilizer. Redirection of B. subtilis metabolism for amino-acid conversion required inactivation of the branched-chain amino-acid (BCAA) global regulator CodY. Additionally, the lipoamide acyltransferase (bkdB) was deleted to prevent conversion of branched-chain 2-keto acids into their acyl-CoA derivatives. With these deletions and heterologous expression of a keto-acid decarboxylase and an alcohol dehydrogenase, the final strain produced biofuels and ammonia from an amino-acid media with 18.9 and 46.6% of the maximum theoretical yield. The process was also demonstrated on several waste proteins.