Addendum

In response to a reader's questions, here we provide additional information about the life cycle analysis (LCA) performed in this paper. The goal of the LCA was to understand the potential environmental benefits of our reported synthetic biology pathways for producing acetone and isopropanol by comparing greenhouse gas emissions with conventional, virgin fossil production routes. Below we expand on the rationale and sources underlying the methodological choices we made in the LCA, including our use of a cradle-to-gate system boundary, an avoided emissions credit, and the descriptor "carbon-negative." We also discuss differences in carbon accounting between an LCA framework and a carbon dioxide removal (CDR) framework.

Cradle-to-Gate System Boundary

Proper LCA methodology requires a system boundary that accurately compares the new pathways to the conventional routes. LCA can be calculated on either a cradle-to-gate or a cradle-to-grave basis. Cradle-to-grave analyses make modelling assumptions on the end-of-life treatment and use of products. In cases where end-of-life is not specified, cradle-to-gate assessments may be more appropriate than cradle-to-grave if the product has a variety of use cases. Isopropanol and acetone have various end-of-life scenarios[1,2], including use as a solvent, where the carbon in the molecule degrades into the environment, or as an intermediate for polymer production, where the carbon could be stored long-term or recycled into other products, achieving circularity. Given the diversity of potential final uses, we presented the LCA on a cradle-to-gate basis, allowing readers the ability to calculate end-of-life scenarios for their specific products.

Our choice of a cradle-to-gate system boundary is supported by multiple sources. ISO 14040:2006 and ISO 14044:2006 outline the guidelines and framework for conducting LCAs[3,4]. ISO 14044 notes that: “Systems shall be compared using the same functional unit and equivalent methodological considerations, such as performance, system boundary, data quality, allocation procedures, decision rules on evaluating inputs, and outputs and impact assessment.” All LCA comparisons between our synthetic biology processes and conventional fossil production routes use the same system boundary and cradle-to-gate assumptions. Importantly, the greenhouse gas savings between our processes and conventionally derived chemicals (on a per kg of acetone or kg of isopropanol basis) would not change between a cradle-to-gate and a cradle-to-grave calculation as end-of-life fates would be applied equally to both. Therefore, use of a cradle-to-gate system boundary provides all necessary calculations to determine the environmental performance of isopropanol and acetone produced from steel mill off-gas.

Several methodologies for performing LCAs of carbon capture and utilization (CCU) technologies, such as LanzaTech’s gas fermentation process, have been developed and are internationally recognized. International Sustainability & Carbon Certification (ISCC) has put forth a Carbon Footprint Certification[5] methodology which provides a detailed approach to calculating product carbon footprints of CCU products. The ISCC Carbon Footprint Certification provides clarity on eligible CCU feedstocks, including biogenic CO_2, direct air capture, and post-industrial gases. ISCC also provides a detailed formula used to calculate product carbon footprints of CCU products at the gate which is consistent with the cradle-to-gate methodology presented in the paper. Additionally, the Roundtable for Sustainable Biomaterials (RSB)[6] supports calculating greenhouse gas emissions at the gate for intermediate products which are products intended to be used in the creation of other materials (e.g. ethylene glycol use in polymer productions), "Operators shall calculate lifecycle cradle-to-gate GHG emissions of certified biomass and/or intermediate products". These certifications represent two of the most common and recognized certification schemes for sustainable chemicals and products.

Avoided Emissions Credit and Carbon Negative Products

In the fuels market, the Renewable Energy Directive (EU)[7], Renewable Transport Fuels Organization (U.K.)[8], and Carbon Offsetting Reduction Scheme for International Aviation (Global)[9] all treat CCU products made from industrial waste off-gases the same way. The CCU product can take credit for the avoided release of the off-gas to the atmosphere so long as the original emitter is still penalized with the release of the gas. These standards also require that the waste gas cannot be purposely generated, meaning that it must be the unintended result of the main production process (i.e., steelmaking in this case). However, it can be difficult for the LCA community to verify this requirement[10,11], especially when utilization of that waste feedstock can result in a new valuable product.

Unlike for fuels, there is not one common approach for the chemicals sector (yet). However, the LCA methodology we used is supported by several guidelines and frameworks that have been developed and recognized by third parties, including government policymakers, research institutions and academia. The Global CO_2 Initiative's *Techno-Economic Assessment & Life Cycle Assessment Guidelines for CO2 Utilization*[12] states that "carbon negative" results are possible at the gate and that cradle-to-gate analyses can be used for a comparative analysis: "…cradle-to-gate analysis, negative LCA results can be computed. However, such negative LCA results only reflect a comparison. In particular, negative LCA results do not necessarily imply that the CCU product is carbon-neutral or even has negative emissions over its entire life cycle.". Similarly, the Together for Sustainability (TfS)[13] methodology for calculating product scope emissions outlines the same carbon accounting methodology for CCU methodologies:~~,~~ “…the cradle-to-gate PCF is provided considering a credit for the avoided waste treatment from the first life cycle”.

Since there is no consensus on whether to take avoided emissions credits in LCA for non-fuel chemicals, and end of life was excluded from the analysis, we performed the LCA with avoided emissions credits. It is the use of avoided emissions credits and a cradle-to-gate boundary that leads to negative carbon intensities of the products. The calculations could change depending on the feedstock and the end-of-life scenario.

We recognize that other methodologies exist. For example, the Environmental Footprint (EF)[14] methodology does not account for carbon uptake, such as atmospheric or avoided industrial carbon release, in carbon footprint calculations. In carbon accounting, this is designated as 0/0, with the first and second numbers representing carbon release and uptake, respectively. The 0/0 approach does not consider avoided emissions (represented as 0 in LCA calculations) but also considers no penalty for its end of life phase, meaning negative carbon intensities for waste and biogenic feedstocks are not possible at the gate though the relative greenhouse gas emissions savings compared to fossil sources would remain the same. It is therefore recommended to perform a cradle-to-grave analysis when using 0/0 methodologies to compare the environmental benefit of chemicals from CCU or biogenic sources to conventional fossil sources. On the other hand, using a +1/-1 approach (the methodology used in this paper) means a credit is taken for carbon uptake (represented as a -1) and a penalty for carbon release (provided by the +1). Therefore, a cradle-to-gate analysis is sufficient to capture the environmental benefits compared to the conventional fossil source.

LCA vs. Carbon Dioxide Removal (CDR) Claims

It should be noted that LCA methodology is different than carbon dioxide removal (CDR) methodology[15]. The main difference is that LCA allows calculation of avoided emissions whereas CDR does not. Thus, LCA allows ‘carbon-negative’ products that do not necessarily mean net atmospheric carbon removal. The LCA in our study includes an avoided emissions credit for steel mill off-gas, which results in the carbon-negative result at the gate. The credit arises from diverting the off-gas from the prior use in which the CO_2 emissions would be released to the atmosphere. This methodological choice means that the emissions of the steel production process do not change, and that the steel production process is burdened with the carbon release to the atmosphere.

Supplementary Table 11 uses steel mill off-gas as an example to highlight carbon accounting and carbon claims using LCA methodology. The example only includes the emissions due to the off-gas feedstock and excludes chemicals or utilities used during processing. The steel mill product will always be considered carbon-positive for fossil carbon sources and carbon-neutral for biogenic sources. The CCU product can be carbon-neutral or carbon-negative depending on the end-of-life (EOL) scenario. In fact, the CCU product is agnostic to the carbon source because the steel mill off-gas is considered a waste in many methodologies.

Supplementary Table 12 provides carbon accounting and carbon claims using the CDR methodology, which considers the the steel mill and CCU products together. In this case, all carbon claims are dependent on the carbon source and EOL scenario. Carbon-negative claims can only be applied to biogenic carbon feedstocks with sequestration EOL scenarios.

Additional LCA Context

We would like to highlight that the gas fermentation process described in the study is agnostic to feedstock as it only requires syngas. Steel mill off-gas was chosen in the LCA as it is the most prevalent commercial application of the gas fermentation technology today[16]. The same LCA methodology would be applied to any accessible waste feedstock with the same gas fermentation process, including solid waste, atmospheric or waste CO_2, and any waste feedstock of biogenic origin. Regardless of the type of waste feedstock chosen, the engineered autotrophic acetogen *Clostridium autoethanogenum* can convert the syngas to isopropanol or acetone using the same gas fermentation technology with lower greenhouse gas emissions than conventional fossil routes when using the system design described in the paper.

Supplementary Table 11: LCA carbon accounting and carbon claims for a hypothetical process that uses 1 kgCO_2 of steel mill off-gas to produce a CCU product. The steel mill producer retains the burden of the off-gas carbon which is consistent with numerous LCA methodologies. +1 implies carbon release while -1 means avoided emissions or carbon sequestration. CCU obtains an avoided emission credit and can be considered carbon -negative or -neutral depending on the EOL scenario. Steel mill off-gas producers can only be carbon -neutral or -positive depending on the EOL scenario. Note that this example only accounts for the off-gas feedstock emissions and does not include emissions due to chemicals or utilities used during processing.

	Fossil Carbon		**Biogenic Carbon**		**Comments**
	Sequestration	**Carbon Release**	**Sequestration**	**Carbon Release**	
LCA Carbon Accounting (kgCO_2)					
Steel Mill Off-Gas Producer	+1	+1	0	0	Steel mill retains the burden of the carbon emissions (RTFO, EU, ISCC, RSB, TFS)
CCU Product (cradle-to-gate)	-1	-1	-1	-1	Avoided emission credit for diverting from prior emissions release
EOL Release by CCU Product	0	+1	0	+1	If carbon is sequestered long-term then no emissions are released
CCU Product (cradle-to-grave)	-1	0	-1	0	Sum of cradle to gate and EOL emissions
LCA Carbon Claims					
Steel Mill Off-Gas Producer	Carbon Positive	Carbon Positive	Carbon Neutral	Carbon Neutral	LCA claims steel mill can make based on carbon source and EOL scenario
CCU Product	Carbon Negative	Carbon Neutral	Carbon Negative	Carbon Neutral	LCA claims CCU product can make based on carbon source and EOL scenario

Supplementary Table 12: CDR accounting and carbon claims of a hypothetical process that uses 1 kg CO_2 of steel mill off-gas to produce a CCU product. CDR considers the combined steel mill producer and CCU product. +1 implies carbon release while -1 means an avoided emission. Fossil carbon sources can only be carbon-neutral or -positive while biogenic carbon can be carbon-negative or -neutral depending on the EOL scenario. Note that this example only accounts for the off-gas feedstock emissions and does not include emissions due to chemicals or utilities used during processing.

	Fossil Carbon		**Biogenic Carbon**		**Comments**
	Sequestration	**Carbon Release**	**Sequestration**	**Carbon Release**	
CDR Carbon Accounting (kgCO_2)					
Steel Mill Off-Gas Producer + CCU Product (cradle-to-gate)	0	0	-1	-1	Negative sources imply the carbon was removed from the atmosphere
EOL Release by CCU Product	0	+1	0	+1	If carbon is sequestered long-term then no emissions are released
Steel Mill Off-Gas Producer + CCU Product (cradle-to-grave)	0	+1	-1	0	Sum of cradle to gate and EOL emissions
CDR Carbon Claims					
Steel Mill Off-Gas Producer + CCU Product	Carbon Neutral	Carbon Positive	Carbon Negative	Carbon Neutral	LCA claims steel mill can make based on carbon source and EOL scenario

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