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Research Article

Carbon Footprint of Biomimetic Carbon Fixation by Immobilizing Nature's CO_2-sequestering Enzyme and Regenerating Its Energy Carrier

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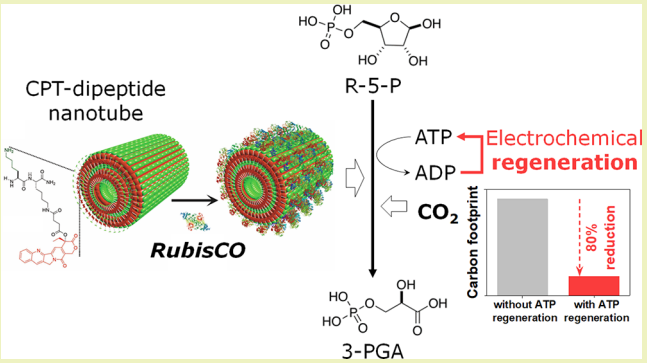
ABSTRACT: In the Calvin cycle of photosynthesis, ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO) catalyzes the conversion of ribulose 1,5-bisphosphate to 3-phosphoglyceric acid (3-PGA) while incorporating atmospheric CO_2 into an organic molecule. Thus, RubisCO is nature's CO_2-sequestering enzyme that is present in chloroplasts. As an effort to mitigate climate change, biomimetic carbon fixation technologies have been developed through RubisCO immobilization into nanostructures to form nanostructure−RubisCO complexes. The technologies mimic the plant cellular environment's ability to convert CO_2 into higher-value products. In this work, a carbon footprint of 3-PGA produced through carbon fixation by the complexes is investigated using the LCA approach. Serine, an amino acid for pharmaceutical applications, is identified as a potential product from 3-PGA. Hotspot processes in terms of the carbon footprint are identified to suggest potential improvements for emerging technologies. Conducting LCA for emerging technologies has many challenges. A sensitivity analysis is performed for uncertain data, and the adenosine triphosphate (ATP) preparation process for the 3-PGA production is identified as a hotspot inventory. We identify that the carbon footprint to produce 3-PGA can be significantly lowered by integrating carbon fixation technologies with an electrochemical ATP regeneration technology.



KEYWORDS: *Life cycle assessment, Emerging technologies, Artificial photosynthesis, Cell-free biomimetics, RubisCO immobilization, ATP regeneration*

INTRODUCTION

In the natural carbon cycle, inorganic CO_2 in the atmosphere is converted to organic hydrocarbons through photosynthesis. However, today's enormous anthropogenic CO_2 emissions along with the increased energy demand make this natural carbon pathway insufficient to close the carbon cycle. Therefore, various CO_2 emissions mitigation strategies are being studied to close this cycle.[1]

One of the strategies is to convert CO_2 into high-value hydrocarbon products such as formic acid, methane, and dimethyl carbonate through chemical conversion processes. Numerous studies have examined the sustainability of such CO_2 conversion technologies.[2] Many of these studies identified environmental effectiveness and technological challenges by conducting life cycle assessment (LCA) studies. Since CO_2 is a stable molecule and requires a huge amount of energy for its activation, CO_2 conversion processes are energy-intensive, and thus, they exhibit positive net CO_2 emissions in many cases.

On the other hand, the carbon cycle of nature (photosynthesis and plant respiration) is closed and attests to the great potential of CO_2 conversion technologies. In the natural carbon cycle, sunlight is a primary energy source for carbon fixation, and it is completely sustainable. To mimic natural photosynthesis, researchers have developed artificial photosynthesis technologies that capture and convert renewable solar energy into high energy density fuels such as hydrogen.[3,4] Renewable energy sources such as solar and wind power, whose emissions are minimal compared to emissions from conventional fossil energy sources, have been considered to reduce the net carbon footprint of CO_2 conversion technologies as well.[2] Alternatively, whole plants can be grown and their biomass can be processed in biorefineries to generate energy and other valuable products. Since biomass

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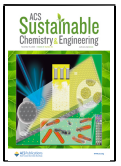

feedstock is prepared through natural photosynthesis, those technologies generally have lower environmental impacts than conventional petrochemical technologies.[5]

More recently, cell-free biomimetic technologies that imitate cellular carbon fixation have been developed.[6] In the Calvin cycle of natural photosynthesis, cascade reactions to produce 3-phosphoglyceric acid (3-PGA) from ribose-5-phosphate (R-5-P) are catalyzed by three enzymes: phosphoribosyl isomerase (PRI), phosphoribulokinase (PRK), and ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). During these reactions, RubisCO, which is an enzyme present in chloroplasts, catalyzes the conversion of ribulose 1,5-bisphosphate (RuBP) to 3-PGA while incorporating atmospheric CO_2 into the organic molecule.[7] RubisCO has been known as the most abundant enzyme in the world and accounts for most of the biological carbon fixation on earth.[8] The biomimetic carbon fixation technologies produce 3-PGA through CO_2 fixation in cell-free systems.[6] In these technologies, three-dimensional nanoscale structures such as nanotubes and nanofibers are employed as support for enzymes to form CO_2-fixing nanostructure-enzyme complexes.[9,10] The nanostructure-enzyme complexes are comprised of three enzymes (PRI, PRK, and RubisCO) and a nanostructure that supports enzyme immobilization. These complexes enhance the catalytic performance of enzymes for cascade reactions.[6]

The cell-free in vitro systems have advantages over in vivo biological systems (living cell systems). Higher product yields and lower environmental impacts are expected from the cell-free systems because of their higher product specificity,[11,12] and cell-free systems do not have to divert resources to other life processes. Also, the CO_2-fixing biomimetic technologies do not require CO_2 capture and compression processes as required by chemical CO_2 conversion technologies. Moreover, compared to biorefinery technologies such as biomass conversion, the cell-free biomimetic technologies avoid the extensive land use for biomass growth and do not impact food production.

The LCA approach is employed to examine the environmental impacts of technologies and identify improvement opportunities. LCA accounts for the life cycle of products which ranges from the extraction of upstream resources to the use and disposal of products. In this sense, LCA is also called a cradle-to-grave analysis. LCA estimates total environmental impacts (e.g., carbon footprint) of products by calculating indirect impacts from the upstream and downstream processes as well as direct impacts. Hotspot inventories that show the highest contribution to the specific impacts could be identified through the LCA study. There are numerous LCA studies on the environmental impacts of chemical CO_2 conversion[2] and biorefinery technologies.[5] However, the environmental effectiveness of cell-free biomimetic carbon fixation technologies remains unknown and the relevant life cycle inventory (LCI) data to such technologies are not readily available from any existing LCI databases. Therefore, challenges and limitations in conducting LCA for such emerging technologies exist and need to be addressed.

In this work, we investigate a carbon footprint of 3-PGA produced through biomimetic carbon fixation by the nanostructure-enzyme complexes using the LCA approach. We investigated two types of nanostructures for the complex as follows:[6,13] Camptothecin (CPT)-dipeptide nanotubes and fluorenylmethyloxycarbonyl (Fmoc) tetrapeptide nanofibers. To examine if the CO_2-fixing 3-PGA has benefits of reducing the footprint, another 3-PGA synthesis route that employs sugar (sucrose) as a carbon source instead of CO_2 is investigated as well.[14] The carbon footprints of three 3-PGA synthesis routes (nanotube route, nanofiber route, and sugar route) are compared with each other. To have the life cycle system boundary, the potential use of 3-PGA is investigated, and the life cycle impacts are calculated. The LCA study identifies potential opportunities for technological improvements to reduce the carbon footprint.

Enzymatic processes generally need the presence of coenzymes such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide (NAD^+). The CO_2-fixing biomimetic technologies also require the use of ATP as the natural photosynthesis in the Calvin cycle does. However, the preparation of these coenzymes is known to be expensive for industrial applications, and therefore, their regeneration techniques have been developed for the economic implementation of enzymatic processes.[15,16] Coenzyme regeneration could reduce wastes and improve the circularity of resources in the enzymatic systems. In this study, we examine if the ATP preparation process is environmentally favorable. Then, we investigate the potential impacts and benefits of integrating an electrochemical ATP regeneration technology into biomimetic carbon fixation systems.

MATERIALS AND METHODS

Nanostructure−RubisCO Complexes. RubisCO is nature's CO_2-sequestering enzyme which catalyzes the conversion of RuBP to 3-PGA. To construct cell-free carbon fixation systems, Satagopan et al. have developed nanostructure−RubisCO complexes.[6] The complexes include three enzymes: PRI, RPK, and RubisCO. PRI and form I RubisCO are prepared from the bacterium *Ralstonia eutropha* as described in the report.[17,18] PRK is prepared from the cyanobacterium Synechococcus as described in the report.[19]

Two types of nanostructures are prepared:[6,13] CPT-dipeptide nanotubes and Fmoc tetrapeptide nanofibers. The synthesis procedures and characterization for these nanostructures and the nanostructure−RubisCO complexes are described in the report.[6] The nanostructure−RubisCO complexes consist of three enzymes and either CPT-dipeptide nanotubes or Fmoc tetrapeptide nanofibers. An illustration of the complex employing CPT-dipeptide nanotubes is included in the Supporting Information (Figure S1). The activity of RubisCO in the complexes has been optimized to its near-native activity levels and the stoichiometric amount of 3-PGA can be produced through these processes.

Use Phase of 3-PGA. 3-PGA is a CO_2-fixed product through the cascade reactions from RuBP in the Calvin cycle. In nature, the Calvin cycle converts 3-PGA to regenerate R-5-P which is converted into ribulose-5-phosphate (Ru-5-P) then RuBP. In the cell-free biomimetic carbon fixation systems, 3-PGA is synthesized by the nanostructure−RubisCO complexes and can be used for industrial applications. According to the report,[20] 3-PGA can be a precursor to synthesize amino acids (serine, cysteine, and glycine) as industrial products for pharmaceutical use. Since serine is a precursor for producing cysteine and glycine, we identify serine as the potential use of 3-PGA in this LCA study.

Serine can be synthesized from 3-PGA through the phosphorylated pathway of serine biosynthesis.[21,22] First, an enzyme 3-phosphoglycerate dehydrogenase (PGDH; *serA*) catalyzes the oxidation of 3-PGA into 3-phospho-hydroxypyruvate (3-PHP). NAD^+/NADH is used as a coenzyme for this conversion. Then, 3-PHP is converted into 3-phospho-serine (3-PS) by using an enzyme phosphoserine transaminase (PSAT; *serC*) and L-glutamate. Finally, 3-PS is hydrolyzed to L-serine using an enzyme phosphoserine phosphatase (PSP; *serB*) and water.

In this work, the following three 3-PGA synthesis routes are investigated.

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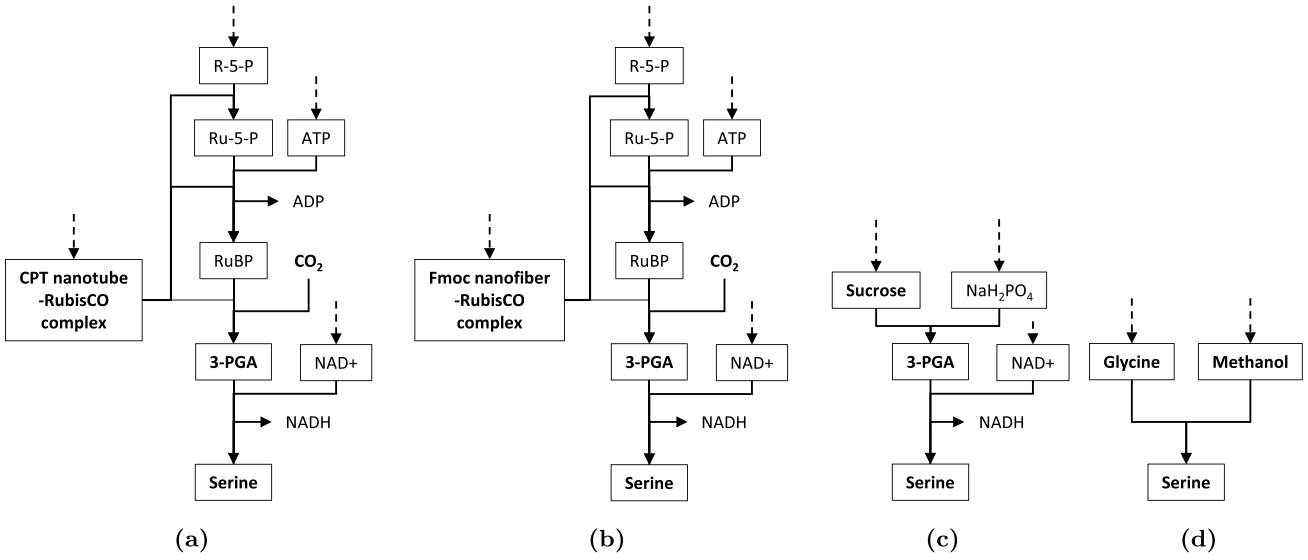

Figure 1. Four routes to produce L-serine: (a) Nanotube route, (b) nanofiber route, (c) sugar route, and (d) conventional route.

- Nanotube route: CPT-dipeptide nanotubes are used as nanostructure support for RubisCO immobilization in the biomimetic CO_2-fixing processes.
- Nanofiber route: Fmoc tetrapeptide nanofibers are used as nanostructure support for RubisCO immobilization in the biomimetic CO_2-fixing processes.
- Sugar route: Sucrose is used as a carbon source instead of CO_2.

3-PGA products from the above routes can be converted into L-serine. Additionally, we examine the conventional L-serine synthesis route. Industrially, serine is synthesized from glycine and methanol by microbial fermentation.[23] The reaction is catalyzed by serine hydroxymethyltransferase (SHMT). Worldwide L-serine production in 2005 was 300 t.[24] Carbon footprints of four serine synthesis routes are investigated using the LCA approach.

ATP Regeneration. In general, enzymatic processes are favorable due to their high selectivity and low environmental impacts. However, many enzymatic reactions require the use of coenzymes such as ATP and NAD^+. The industrial use of coenzymes is impractical because their preparation processes are expensive. Therefore, the regeneration of coenzymes has been the limiting step for their economic utilization.[15,16]

In this study, an electrochemical ATP regeneration technology is considered as a means of reusing coenzymes and reducing environmental impacts. Electrochemical processes can directly utilize electrons, instead of using reductants and oxidants, by controlling electrode potential to achieve specific reactions.[25] A programmable chemical actuator was created and programmed to control the inorganic phosphate concentration at the electrode surface, condensing ADP to ATP in situ. Working and counter electrodes (0.064 cm^2) were prepared from Au wire (0.05 mm radius) and a 0.064 cm^2 Ag/AgCl reference electrode was prepared with Ag wire (0.01 mm radius). A total of 253 μg of polypyrrole doped with sodium chloride (PPy(Cl)) was electropolymerized on the working electrode and then programmed for phosphate selectivity in 0.5 M Na_3PO_4. The integration of the ATP regeneration system with the biomimetic CO_2 fixation system means that the same reaction medium is employed for the CO_2 fixation by the nanostructure–RubisCO complexes and for the electrochemical ATP regeneration. The following stoichiometric reaction is assumed for ATP regeneration from ADP and inorganic phosphate:

$$ADP + H_3PO_4 \rightarrow ATP + H_2O \quad (\Delta G^\circ = 30.5\ kJ/mol) \qquad (1)$$

The PPy(PO_4^{3-}) membrane is prepared through a two part electrochemical process using 0.1 M pyrrole (Py) monomer, 0.1 M NaCl as a dopant, and 0.5 M Na_3PO_4 as an equilibration solution. First, the Py monomer and NaCl are oxidized at the working electrode, then the polymerization solution is exchanged for the equilibration solution and cyclic voltammetry is performed to form ion-selective pathways in the polymer. ADP is condensed to ATP when the polymer is oxidized, and the concentration of inorganic phosphate is raised at the electrode surface. Polymerization of PPy(Cl), programming of PO_4^{3-} selectivity, and the operation of systems consume 2.92, 0.34, and 25.47 J of energy, respectively. The phosphate actuator has a lifetime of 1,200 cycles before the polymer must be replaced or refreshed. More details about the mechanism for electrochemical ATP regeneration are described in the Supporting Information (Figures S2–S5).

Life Cycle Assessment. LCA is a tool to assess the environmental impacts of products and processes by accounting for their upstream and downstream life cycle activities. In this study, an open-source LCA software (openLCA)[26] is used to conduct the LCA study. The LCA approach is documented in ISO 14040:2006 and consists of four phases[27] that are described in the context of the selected technology in the following subsections.

Goal and Scope Definition. The goal of the LCA study is to examine how effective biomimetic carbon fixation technologies are to mitigate global warming and to identify how the technologies could be further improved. The analysis boundary is a cradle-to-gate boundary (cradle-to-use phase) which ranges from the raw material extraction to the use phase of 3-PGA. When the same two products with different upstream technologies are investigated, a cradle-to-gate analysis is performed since the downstream activities (use and disposal phases) for the common product are identical to each other. L-Serine is identified as the potential use of 3-PGA. Figure 1 shows four types of product systems investigated in this study. Nanotube and nanofiber routes are the L-serine synthesis routes from 3-PGA, which is prepared through carbon fixation by nanostructure–RubisCO complexes. Sugar route is the L-serine synthesis route from 3-PGA, which is prepared using sucrose as a carbon source instead of CO_2. The sugar route is included in the study to compare the biomimetic carbon fixation technologies to a non-CO_2-fixing technology. Lastly, the conventional route to L-serine involves the microbial fermentation of glycine and methanol. Accordingly, a function unit is defined as 1 kg of L-serine.

Inventory Analysis. In this work, two existing LCI databases (U.S. LCI database and Ecoinvent) are employed. The U.S. LCI database is prioritized when the desired inventory data is available since the database is based on the U.S. Applying LCA to emerging technologies is challenging because many of their inventory data are difficult to find in any LCI database due to their nascent nature.[28] In such cases, any data that are not available in existing databases are either estimated

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from laboratory experiments or obtained from literature such as journal articles and patents. If industrial production data is available, it is preferred. Such LCA studies of emerging technologies are likely to have large uncertainty. To account for this uncertainty, a sensitivity analysis is performed on unknown inventory data.

Some data are not available even from the literature. In such a case, the data are estimated by simple fundamental models or ignored in the study. For example, any catalyst inputs are ignored assuming their reusability and small quantity. The manufacture of equipment and the transportation of materials are also excluded unless such data are available from the LCI databases or literature. Moreover, if data for the energy inputs are not provided, they are excluded. This implicitly assumes that these processes occur around room temperature, which is true for most biological processes. Furthermore, product yields are determined by stoichiometric calculation if such data are unknown.

Figures S6−S9 in the Supporting Information show partial life cycle network diagrams for the nanotube, nanofiber, sugar, and conventional routes to produce L-serine, respectively. The complexity of the diagrams for the nanotube and nanofiber routes implies difficulties in applying LCA to emerging technologies because their inventory data are difficult to find in any LCI database. Adenosine diphosphate (ADP) from the conversion of Ru-5-P to RuBP and NADH from the conversion of 3-PGA to 3-PHP are considered to be waste since these are the cofactors that need to be regenerated into ATP and NAD+, respectively. They also inhibit the reactions using ATP and NAD+ when present in high amounts.

The LCA studies can specify inventories that contribute the most to the impacts in the life cycle system boundary. In this LCA of emerging technologies, the opportunities for technological improvements can be identified for the inventories whose data are based on experiments, instead of commercialized processes (i.e., the inventories in the green dotted box in Figures S2 and S3). Therefore, either of the following opportunities can be identified:

- Improving the yield of product or discovering an alternative technology to prepare a reactant, if any reactant inventory is identified as a hotspot inventory.
- Improving the efficiency of solvent use or replacing it with the alternative one that has smaller impacts, if any solvent inventory is identified as a hotspot inventory.

If we consider the integration of electrochemical ATP regeneration technology into the carbon fixation technologies, the analysis boundary needs to include the ATP regeneration process. In such a case, ADP from the cascade reactions is not a waste anymore. Instead, ADP is recycled into ATP through regeneration. Figure 2 shows the partial life cycle network diagram for the electrochemical ATP regeneration. Sources of inventory data are indicated in different colors. The diagram does not show the upstream network for the inventories available in the existing LCI databases. The vertical and horizontal input arrows represent reactants and solvent use, respectively. The output arrows indicate products. The purple dotted box indicates the boundary of ATP regeneration technology. The electrochemical experiments are performed to collect the inventory data in the purple dotted box (Tables S10 and S11 of the Supporting Information). PPy(PO_4^{3-}) membrane is prepared from the pyrrole monomer through electrochemical polymerization. ATP is regenerated from ADP through the electrochemical procedure. ADP is provided from the biomimetic carbon fixation, and the regenerated ATP is used in the carbon fixation process.

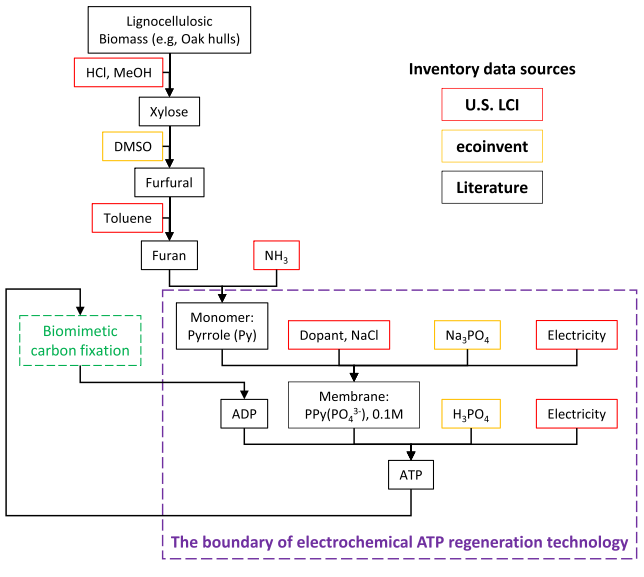

Figure 2. Network diagram for the electrochemical ATP regeneration.

In many LCA studies on electrolysis in a membrane cell, electrodes and membranes are excluded from the analysis since they are assumed to be reusable.[29] However, the membrane is included in this LCA study since it is known that the polypyrrole membrane can be reused more than 1,200 times.

Impact Assessment. A carbon footprint of four routes to produce serine is calculated. Life cycle interventions are characterized using TRACI 2.1 life cycle impact assessment (LCIA) method.[30] If byproducts and coproducts are produced from a certain inventory *A*, the impacts need to be allocated to the main product. The allocation needs to be performed by either the displacement method or the partitioning method. The former method considers a conventional way of producing those byproducts. Then, the byproducts are assumed to replace the conventional process. Therefore, the impacts from the conventional process are avoided due to the byproducts produced from the inventory *A*. In the partitioning method, the impacts from the inventory *A* are partitioned among the main product and byproducts based on the ratio of mass, energy, or monetary values. The displacement approach is preferred if conventional process data are available from the LCI databases or literature.

Interpretation. Hotspot inventories (i.e., the largest contributors to the carbon footprint) can be identified through LCIA. Opportunities for technological improvements to reduce the footprint can be discussed. In the following section, we compare the carbon footprint of four serine synthesis routes. Also, we discuss how effectively the coenzyme regeneration technology can reduce the footprint of biomimetic CO_2 fixation technologies.

RESULTS AND DISCUSSION

LCA of Biomimetic Carbon Fixation without ATP Regeneration. A cradle-to-gate LCA study is performed on biomimetic carbon fixation technologies to investigate a carbon footprint of technologies. These emerging technologies produce 3-PGA product through carbon fixation by nanostructure−RubisCO complexes. In this LCA study, the following two types of nanostructures are investigated: CPT-dipeptide nanotubes and tetrapeptide nanofibers.

In the sensitivity analysis, three cases (lower impact, base, and higher impact) are considered for the ratio of unreacted reactants to be reused, the ratio of fugitive volatile organic compound (VOC) emissions, the number of times of solvent reuse, and the number of times the nanostructure−RubisCO complex is reused. Table 1 summarizes three cases considered via the sensitivity analysis. The lower impact case represents a more environmentally beneficial case while the higher impact case refers to the less beneficial case.

80−100% of unreacted reactants are considered to be reused. The unreacted materials that are not reused are assumed to be emitted to the air if they are in the gas phase at room temperature (e.g., ammonia and phosgene). VOC emissions are the fugitive air emissions from the use of VOCs. They include fugitive emissions from solvents,

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Table 1. Three Cases Considered via a Sensitivity Analysis

description	lower impact	base case	higher impact
unreacted reactants to be reused (%)	100	90	80
fugitive VVOC emissions (%)	2	4	8
fugitive VOC emissions (%)	1	2	4
fugitive SVOC emissions (%)	0.5	1	2
solvents to be reused (times)	20	10	5
complex to be reused (times)	20 000	10 000	5000

byproducts, and unreacted reactants. 0.5−8% of VOCs are assumed to be lost to the air as fugitive emissions. The VOCs can be classified into three categories based on the boiling point range: VVOC (very VOC), VOC, and SVOC (semi-VOC).[31,32] In this study, the organic compounds are VVOC, VOC, or SVOC, if their boiling points range from the room temperature to 75 °C, from 75 to 250 °C, or from 250 to 400 °C, respectively. Every solvent in this study is assumed to be reused 5−20 times through the sensitivity analysis.[33] Also, the nanostructure−RubisCO complexes can be reused numerous times since they show enzymatic behavior. Based on input from the technology developers, the number of times of reuse of this complex is considered to be between 5000 and 20 000.

Figure 3a exhibits the LCIA results of four serine synthesis routes for three sensitivity analysis cases. A total carbon footprint to produce 1 kg of serine is shown at the top of each bar. The carbon footprint for preparing 3-PGA is shown in green bars. The contribution to the footprint from solvent use ($NaHCO_3$, Tris, HCl) and the other reactant use (NAD+, L-glutamate) for the serine synthesis from 3-PGA is plotted as well.

As shown in Figure 3a, the conventional route exhibits the lowest carbon footprint followed by the nanofiber, nanotube, and sugar routes. The sensitivity analysis indicates the results are robust since all sensitivity cases show the same relative results. For each sensitivity case, the footprint from solvent use ($NaHCO_3$, Tris, HCl) and reactant use (NAD+, L-glutamate) is the same among three routes from 3-PGA (nanotube, nanofiber, and sugar routes). For instance, the footprint from the use of NAD+ is 96.7 $kgCO_2eq$/kg-serine for those three routes in the base case. This is because those routes share the same use phase of 3-PGA. Also, the impacts of these three routes are very sensitive to the cases considered in the sensitivity analysis. The footprint from using NAD+ varies from 15.6−240.1 $kgCO_2eq$/kg-serine depending on the sensitivity cases. This is due to the nascent nature of technologies for preparing NAD+ as well as for converting 3-PGA to 3-PHP using NAD+. Since the technologies are not yet fully developed, the inventory data are unavailable from the LCI databases and the impacts of the technologies for industrial scale are uncertain.

In the conventional route, the most dominant inventory in the footprint is methanol which accounts for 85.4−88.4% of the total footprint. Sensitivity analysis shows that uncertainty in the carbon footprint from the conventional route is very small compared to that from the other three routes. This is because, unlike emerging technologies, most of the inventory data for the conventional serine synthesis technology are available from the LCI databases. This indicates that most technologies in the conventional route have already been optimized for commercial scale. Therefore, the impacts of the conventional route do not vary a lot with the sensitivity cases.

The biomimetic carbon fixation technologies in the nanotube and nanofiber routes have a lower carbon footprint than the non-CO_2-fixing technology in the sugar route. The nanotube and nanofiber routes show 18.5−35.4% and 20.3−35.8% lower footprint than the sugar route, respectively, depending on the sensitivity cases. However, both biomimetic routes show a much higher footprint than the conventional route. These biomimetic technologies are still in development and need to be further improved to be effective in mitigating

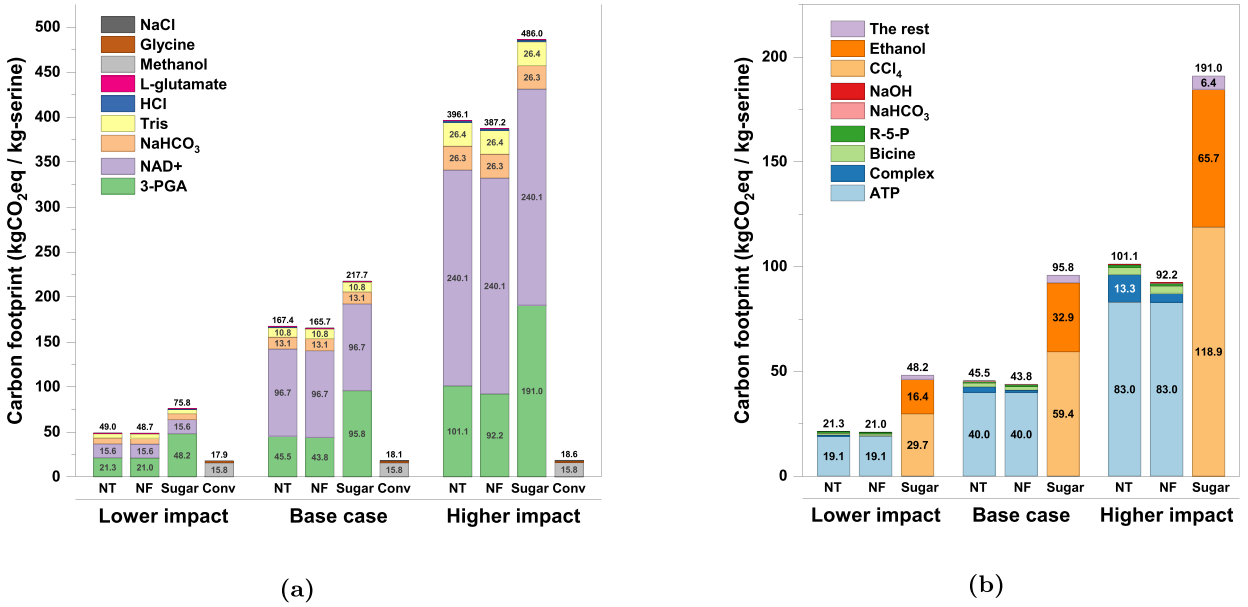

Figure 3. (a) Life cycle impact assessment results of four routes to produce serine. A total carbon footprint to produce 1 kg of serine is shown at the top of each bar. Biomimetic nanotube (NT) and nanofiber (NF) routes have lower footprints than the sugar route but higher footprints than the conventional route. (b) Detailed LCIA results for 3-PGA production in three serine synthesis routes. ATP inventory shows the largest contribution to the carbon footprint of biomimetic NT and NF routes.

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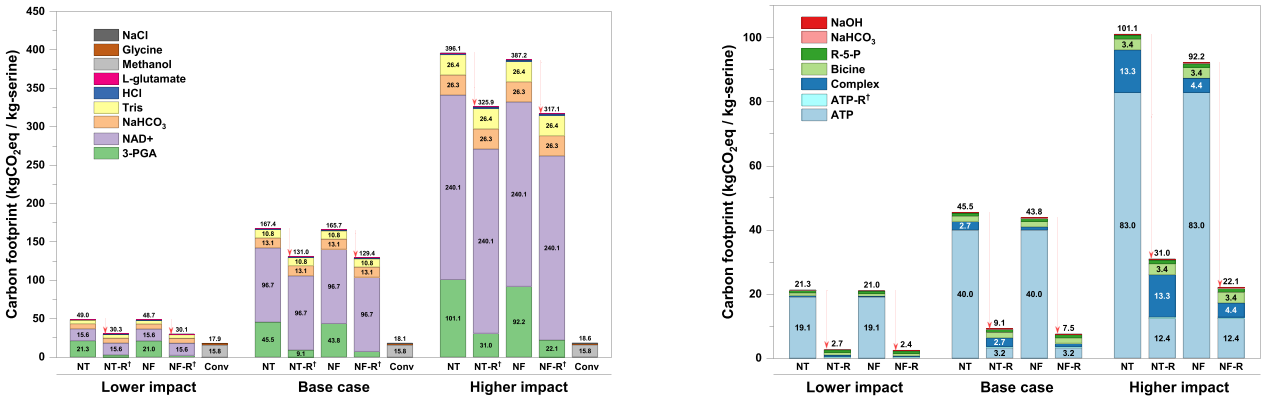

Figure 4. (a) Comparison of LCIA results among the nanotube (NT), nanofiber (NF), and conventional routes to produce serine. A carbon footprint of biomimetic NT and NF routes is reduced by integrating ATP regeneration as shown in red-colored dotted arrows. †NT-R and NF-R refer to the nanotube and nanofiber routes, respectively, that include ATP regeneration. (b) Detailed LCIA results for ATP-regenerated 3-PGA production between the NT and NF routes. †ATP-R represents electrochemical ATP regeneration inventory.

global warming. The opportunities for improvements can be identified through the LCA study.

Figure 3b compares three routes to produce serine from 3-PGA in more detail. A carbon footprint of producing 3-PGA in the nanotube, nanofiber, and sugar routes is calculated. For the biomimetic carbon fixation technologies, ATP inventory dominates the carbon footprint. 82.0−89.6% and 89.9−91.3% of the footprint to produce 3-PGA are attributed to the ATP process in the nanotube and nanofiber routes, respectively. ATP is used for the phosphorylation of Ru-5-P to RuBP as part of the Calvin cycle. ATP is converted to ADP by providing phosphate to the Ru-5-P molecule.

The nanofiber route has a lower footprint than the nanotube route because the CPT-dipeptide nanotubes have a higher footprint than the Fmoc tetrapeptide nanofibers. To prepare CPT-dipeptide, CPT-succinic acid is used. The isolation of CPT is a carbon-intensive process with low yield.[34] Despite the fact that CPT nanotubes have a high footprint, the CPT nanotube-RubisCO complex only accounts for 2.2−13.1% of the footprint to produce 3-PGA. In case of the Fmoc nanofiber-RubisCO complex, it accounts for 1.0−4.8% of the footprint to produce 3-PGA. This small contribution to the footprint is attributed to the complexes' reusability due to their enzymatic behavior in the cascade reactions to produce 3-PGA.

In case of the sugar route, the main contribution to the footprint is the use of carbon tetrachloride (CCl_4), which is one of the major global warming substances. CCl_4 has a 100-year global warming potential of 1,730 $kgCO_2eq/kg\text{-}CCl_4$[35] and is highly toxic. In recent years, its use and emissions have been in decline.[36]

For the base case of the nanotube route, the production phase of 3-PGA and its use phase for serine account for 27.4 and 72.6% of the total footprint, respectively, as indicated in Figure S10 of the Supporting Information. The results identify the preparation of coenzymes such as ATP and NAD+ as hotspot inventories with respect to the carbon footprint. In the production phase, the ATP preparation process shows the highest contribution to the footprint. In the use phase, the NAD+ preparation process contributes the most to the footprint. Those coenzyme preparation processes have low production yields and require the use of a variety of solvents. Coenzyme preparation has been known as economically expensive.[15,16] The LCA results imply that coenzyme preparation is also environmentally unfavorable.

To improve the biomimetic carbon fixation technologies, the burden of preparing ATP needs to be reduced. Considering that 3-PGA product yield from the cascade reactions is already close to the stoichiometric level, an alternative pathway for preparing ATP needs to be investigated to reduce the footprint of technologies.

LCA of Biomimetic Carbon Fixation Integrating ATP Regeneration. The LCA study in the previous section identifies the coenzyme preparation processes to be the main contributors to the carbon footprint of biomimetic carbon fixation technologies. In this section, a carbon footprint of the carbon fixation processes including ATP regeneration is investigated. Figure 4a compares the LCIA results between the original systems and the ATP-regenerated systems for the nanotube, nanofiber, and conventional serine synthesis routes. A sensitivity analysis is performed for three cases.

Even though ATP is regenerated from ADP, some portions of ATP still need to be prepared from adenosine for the base and higher impact cases because the excess amount of ATP is required for the biomimetic carbon fixation technologies. In the cascade reactions, one mole of ATP is required to produce two moles of 3-PGA. In the experiments, however, 15 mol of ATP are added in producing 16 mol of 3-PGA. Since the stoichiometric formation of 3-PGA is confirmed in the experiments,[6] 7 mol of ATP can be considered to be unreacted. For the base and higher impact cases, 10 and 20% (0.7 and 1.4 mol) of unreacted materials, respectively, are assumed to be emitted as waste. Hence, even if we consider the stoichiometric regeneration of ATP from ADP, 0.7 and 1.4 mol of additional ATP need to be prepared from adenosine for the base and higher impact cases, respectively.

As shown in Figure 4a, a carbon footprint for producing serine is reduced by 21.7% for the nanotube route and 21.9% for the nanofiber route in the base case. In the lower impact case, the ATP-regenerated, CO_2-fixing serine synthesis technologies (NT-R and NF-R routes) show the most competitive footprint to the conventional serine synthesis technology (conventional route). However, the footprint of the

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carbon fixation technologies is still higher than the conventional route for every sensitivity case. The main contributor to the footprint of the carbon fixation technologies is the use of NAD+ coenzyme for serine synthesis. This implies that the footprint could potentially be decreased significantly if the NAD+ coenzyme is also regenerated from NADH by employing another chemical actuator. Also, the power to run chemical actuators can be negligible if this comes from renewable sources. In this study, NAD+ regeneration is not considered due to a lack of experimental data. The ATP regeneration reduces the footprint of the ATP inventory by 97.6, 90.8, and 84.6% for the lower impact, base, and higher impact cases, respectively. If we assume a similar reduction in the footprint for the NAD+ regeneration, 13.3−40.0 $kgCO_2$/kg-serine of the total footprint is expected for the nanofiber route. In the lower sensitivity case, this footprint (13.3 $kgCO_2$/kg-serine) is smaller than the conventional route (17.9 $kgCO_2$/kg-serine).

The detailed LCIA results for ATP-regenerated 3-PGA production between the nanotube and nanofiber routes are shown in Figure 4b. ATP regeneration reduces the footprint dramatically since it has a significantly smaller footprint compared to the ATP preparation process from adenosine. nanostructure−RubisCO complex inventories are identified as new hotspot inventories in terms of the carbon footprint to further improve the carbon fixation technologies. Most of the footprint for the complexes comes from nanostructure supports. Fmoc nanofibers have a smaller footprint than the CPT nanotubes. Other nanostructures as support for enzyme immobilization could be investigated as well. For instance, cellulose nanofibers and chitin nanofibers show high enzymatic activity and stability when they are incorporated with enzymes into the nanostructure-enzyme complex.[37,38] Also, cellulose and chitin are cheaper resources compared to CPT and Fmoc. Further studies are needed for biomimetic carbon fixation to find the most effective nanostructure candidate as support for RubisCO immobilization.

CONCLUSIONS

This work describes the carbon footprint of biomimetic carbon fixation technologies that employ a RubisCO immobilization technique. RubisCO is immobilized into either CPT-dipeptide nanotubes or Fmoc tetrapeptide nanofibers to form the nanostructure−RubisCO complexes. The complexes catalyst cascade reactions from R-5-P to 3-PGA while fixing CO_2 into the organic 3-PGA molecule. 3-PGA then can be used to synthesize L-serine, an amino acid for pharmaceutical use. The footprint of the technologies was compared with a non-CO_2-fixing technology and the conventional serine synthesis technology. The non-CO_2-fixing technology uses sugar as a carbon source instead of CO_2 to produce 3-PGA. The conventional technology synthesizes L-serine from methanol and glycine, not from 3-PGA.

A sensitivity analysis was performed to account for uncertainty in the inventory data for emerging technologies. The biomimetic carbon fixation technologies showed a lower carbon footprint than the non-CO_2-fixing technology. However, their footprint was much higher than conventional technology. The LCA study identified the preparation processes of coenzymes such as ATP and NAD+ as hotspot inventories in terms of the carbon footprint. ATP is used in the cascade reactions to produce 3-PGA while NAD+ is used to produce serine from 3-PGA.

To reduce the footprint of the carbon fixation technologies, integrated systems of electrochemical ATP regeneration and biomimetic carbon fixation were considered. We performed LCA for the integrated systems. The carbon footprint of emerging technologies to produce serine was decreased by 17.7−38.3% with the regeneration of ATP from ADP. We identified that the carbon footprint of the emerging technologies could potentially be lower than the conventional technology if NAD+ could be regenerated from NADH in a similar manner to the ATP regeneration. The LCIA results for the biomimetic carbon fixation technologies indicated that coenzymes need to be regenerated in order to lower the footprint below that of the conventional process. To further improve the technologies, other types of nanostructures could be examined as support for RubisCO immobilization to see if they lead to better LCIA results.

The analysis boundary of this LCA study was cradle-to-gate (cradle-to-use phase). The nanostructure−RubisCO complexes are assumed to be reusable 5,000−20,000 times. For a more complete LCA study, the disposal phase could be included to account for the impacts of waste treatment. Therefore, we need to investigate how the downstream processes of the complexes affect the overall carbon footprint. Currently, such experimental data are not available. In addition, impacts besides carbon footprint should also be considered.

Given the nascent nature of technologies, many challenges remain in conducting LCA for emerging technologies. Early stage experimental data will not represent commercialized data since inventory data could highly depend on the production scale. In this study, we performed a sensitivity analysis to account for those uncertain data gaps. However, it is important to perform a robust LCA study using more realistic and data that is closer to the use of mature technologies at an industrial scale.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssuschemeng.0c05498.

Illustration of the nanostructure−RubisCO complex, details about the electrochemical ATP regeneration, partial life cycle network diagrams to produce L-serine, and an additional supporting figure for the Results and Discussion section (PDF)

Life cycle inventory data for experimental processes (XLSX)

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Author Contributions

K.L. developed the LCA model, conducted the carbon footprint analysis, and took the lead in writing the manuscript. Y.S., S.S., and J.P.E. performed the experiments for RubisCO immobilization, biomimetic carbon fixation, and electrochemical coenzyme regeneration, respectively, and provided experimental process data for the LCA model. J.P.E. wrote the ATP regeneration section of the manuscript. J.R.P., F.R.T., and V.B.S. guided the experimental work. K.L., S.S., F.R.T., J.R.P., and B.R.B. discussed and proposed the use phase of 3-PGA. B.R.B. guided the LCA work and edited the manuscript. All authors provided critical feedback and contributed to the final version of the manuscript. J.R.P. and B.R.B. supervised the project.

Notes

The authors declare no competing financial interest.

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