Powering Carbon-Optimized Bioconversions

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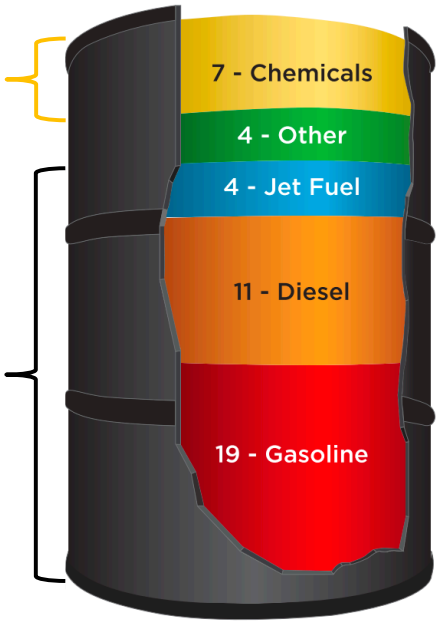
ARPA-E Workshop on "Carbon-Optimized Bioconversion", Chicago, September 26-27, 2019

Petroleum products and "bio-equivalents"

Chemicals make up **16% of the volume** of U.S. oil products and are worth $812 billion



Bioproducts to Enable Biofuels Workshop Summary Report, DOE 2015

Fuel makes up **76% of the volume** of U.S. oil products and is worth $935 billion

Degree of reduction (DOR): # of electrons available per carbon

DORs: Petroleum = 5.8-6.0; CH_4 = 8; $C_6H_{12}O_6$ = 4; Lignin = 4.5-4.7; CO_2 = 0

Petroleum products	DOR	"Bio-equivalent"
Gasoline, Diesel, Jet fuel, Methanol, Ethylene, Propylene, Butadiene, BTX	6	Ethanol, Butanol, Fatty acid methyl ester (FAME)
	5	1,3-PDO, 1,4-BDO, Xylitol, Arabinitol, Sorbitol
	4	Lactic acid, Glucose, Acetic acid, Succinic acid
	3	FDCA, Malic acid, Fumaric acid

DOR = 4*# carbons + 1*# hydrogens – 2*# oxygens/# carbons

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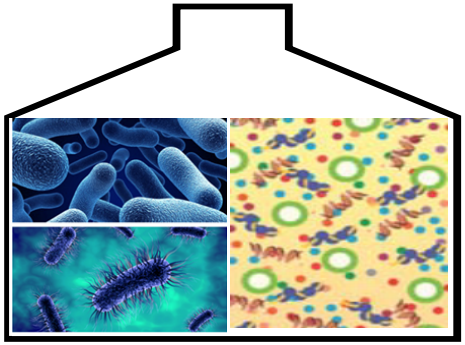
Bioconversions are "typically" optimized for maximum energy efficiency

Assuming Deconstruction & Fractionation via Pretreatment/Preprocessing & Hydrolysis: Sugars, Lignin/Aromatics, etc.

Glucose ($C_6H_{12}O_6$)
C&E Source
DOR: 4



Alcohol
C-based Product
DOR: 6

$$\text{Glucose } (C_6H_{12}O_6) \longrightarrow \text{Butanol } (C_4H_{10}O) + \boxed{2CO_2} + H_2O$$

$\Delta G° = -265.9$ kJ/mol
C efficiency: 67%
E efficiency: 97%

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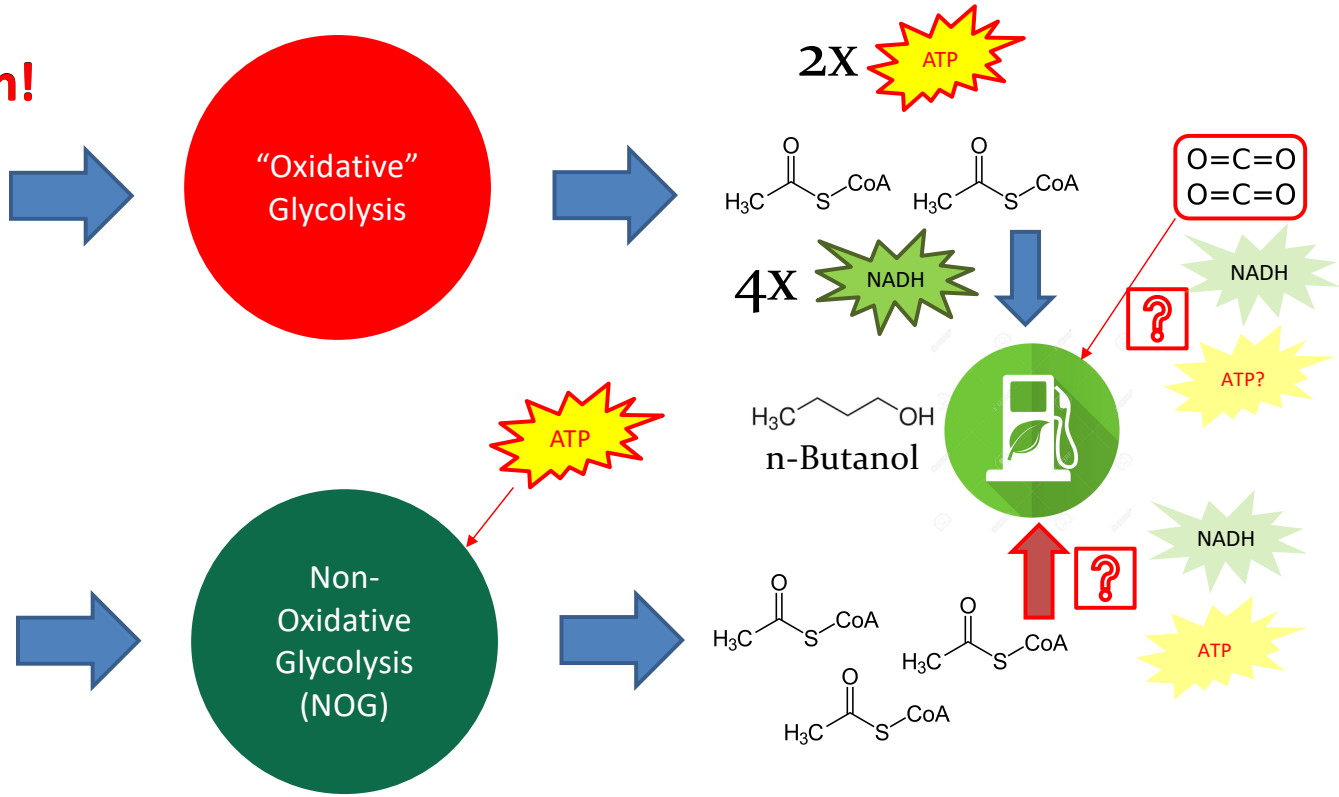
Maximizing C efficiency results in energy limitations/requires extra energy

Houston, we have (*an energy*) problem!

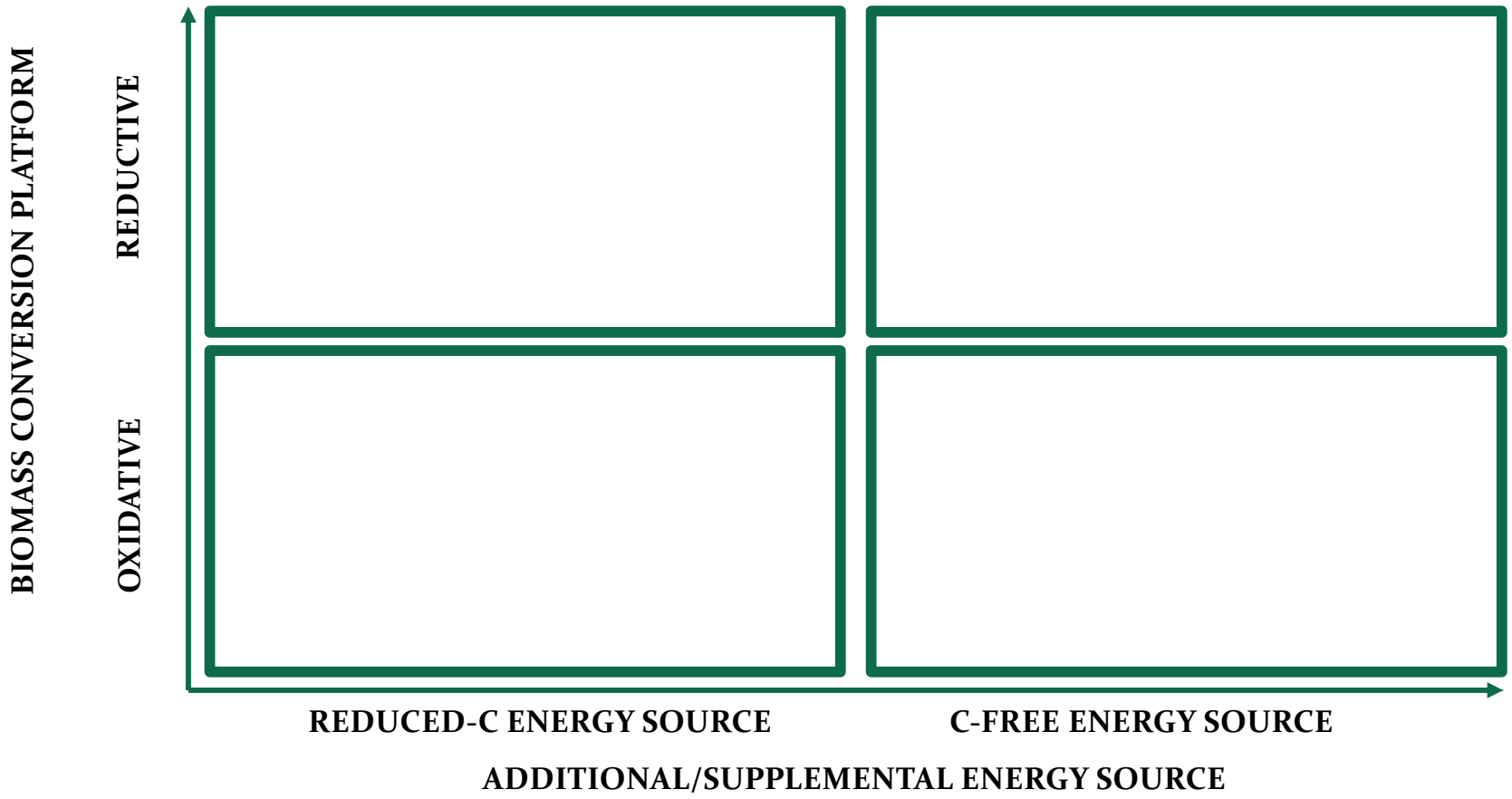
Glucose ($C_6H_{12}O_6$)
C&E Source
DOR: 4

Where do we get that energy from?

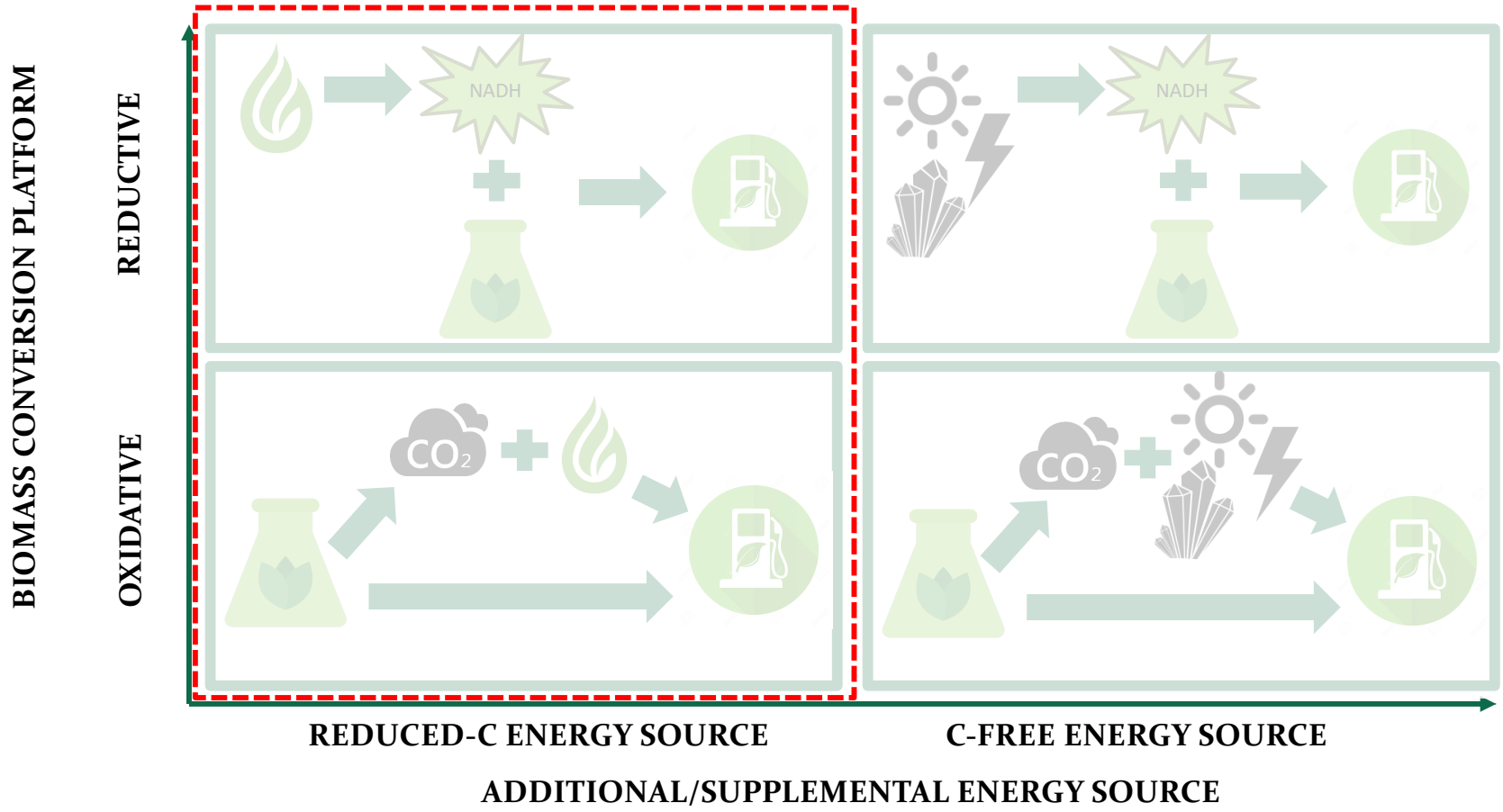


Liao's Laboratory: *Nature,* 2013: 502:693–697; *PNAS: 2018 115 3538-3546*

Conversion platform & source of supplemental energy



Conversion platform & source of supplemental energy



Higher C efficiency requires feedstock with higher DOR

Carbon source	DOR	Reaction (oxygen independent)	ΔG° (kJ/mol)	C efficiency
Sugar	4.00	$C_6H_{12}O_6 \longrightarrow C_4H_{10}O + 2CO_2 + H_2O$	-256.9	67%
Glycerol*	4.67	$2\ C_3H_8O_3 \longrightarrow 1.17\ C_4H_{10}O + 1.33\ CO_2 + 2.17\ H_2O$	-244.6	78%
Palmitic acid (C16)**	5.75	$3/8\ C_{16}H_{32}O_2 + 1.19\ H_2O \longrightarrow 1.44\ C_4H_{10}O + 0.25\ CO_2$	53.2	96%
Methane	8.00	$6\ CH_4 + 2\ CO_2 \longrightarrow 2\ C_4H_{10}O + 2\ H_2O$	157.9	133%

**Dharmadi, Murarka, Gonzalez, 2006. B&B 94: 821; Clomburg, Gonzalez, 2013, TIBTECH 31: 20; **Dellomonaco, Rivera, Campbell, Gonzalez, 2010, AEM 76: 5067*

Glucose (DOR=4) Reduced carbon (DOR>6) n-Butanol (DOR=6)

$$\text{Glucose } (C_6H_{12}O_6) + 6 \text{ Methane } (CH_4) \longrightarrow 3 \text{ Butanol } (C_4H_{10}O) + 3H_2O \qquad \Delta G° = -107.9 \text{ kJ/mol}$$

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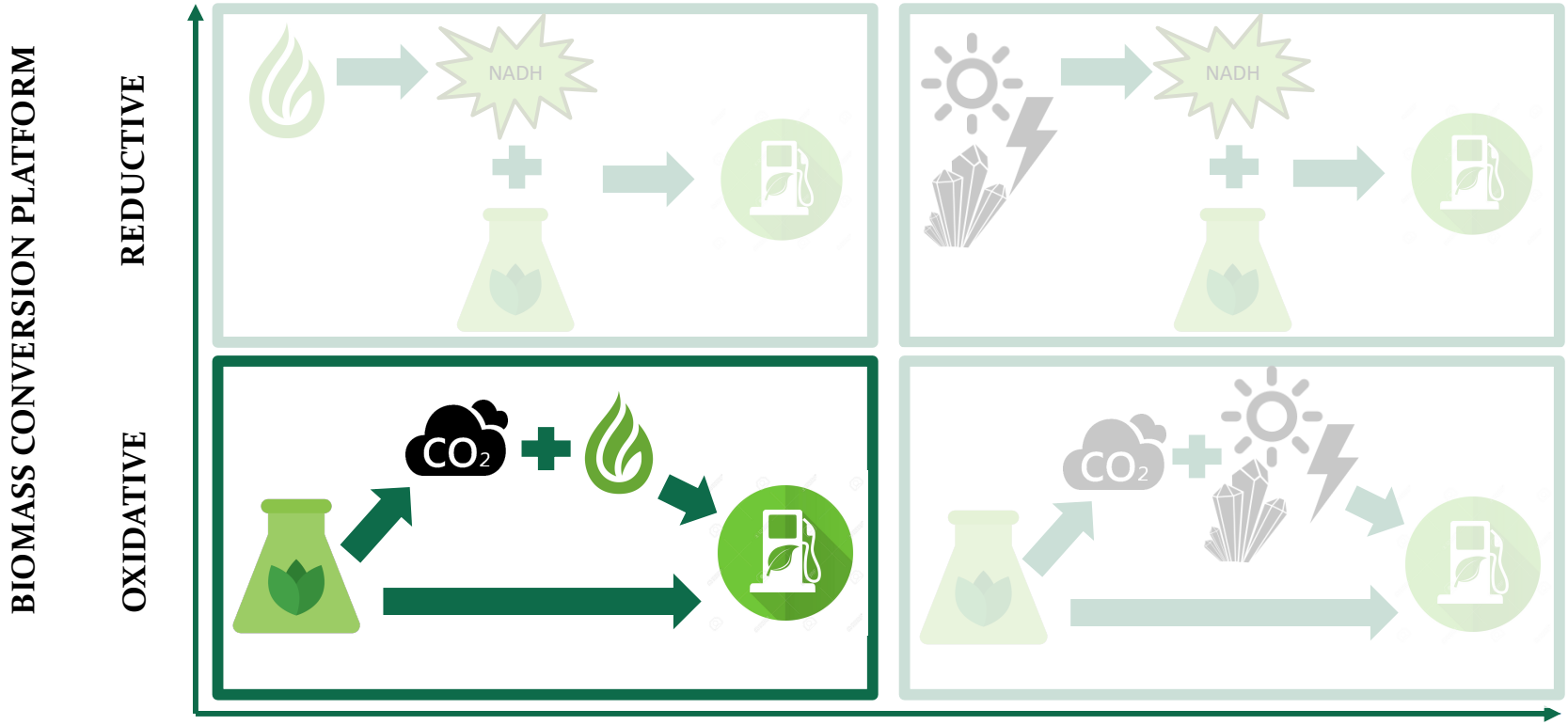
"Oxidative" conversion platform & "reduced-C" energy



ADDITIONAL/SUPPLEMENTAL ENERGY SOURCE

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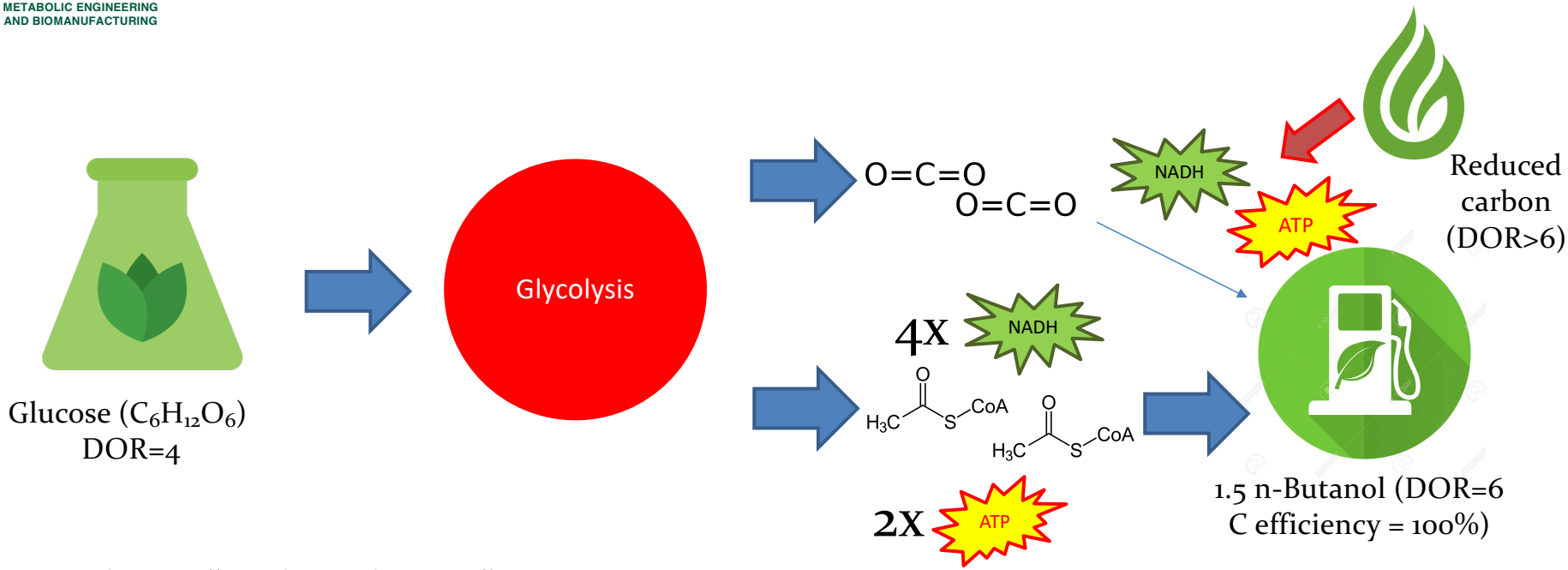
"Reduced-carbon" energy source for CO2 fixation



Methane "carboxylation"

Methane (CH_4) + CO_2 + CoA ⟶ Acetyl-CoA + H_2O $\Delta G° = 41$ kJ/mol

3 Methane (CH_4) + CO_2 ⟶ Butanol ($C_4H_{10}O$) + H_2O $\Delta G° = 79$ kJ/mol

ATP?

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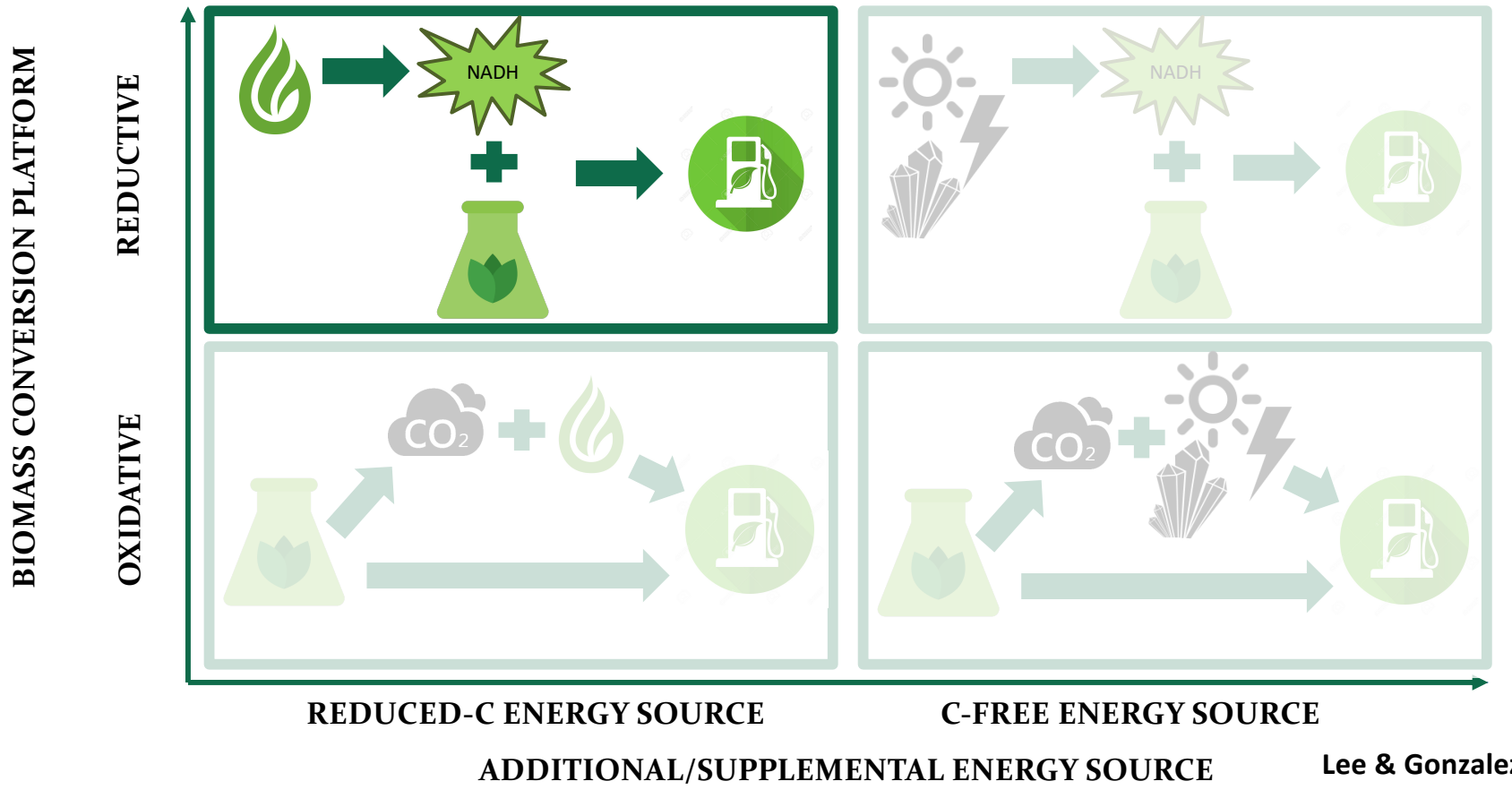
“Non-oxidative” conversion & “reduced-C” energy



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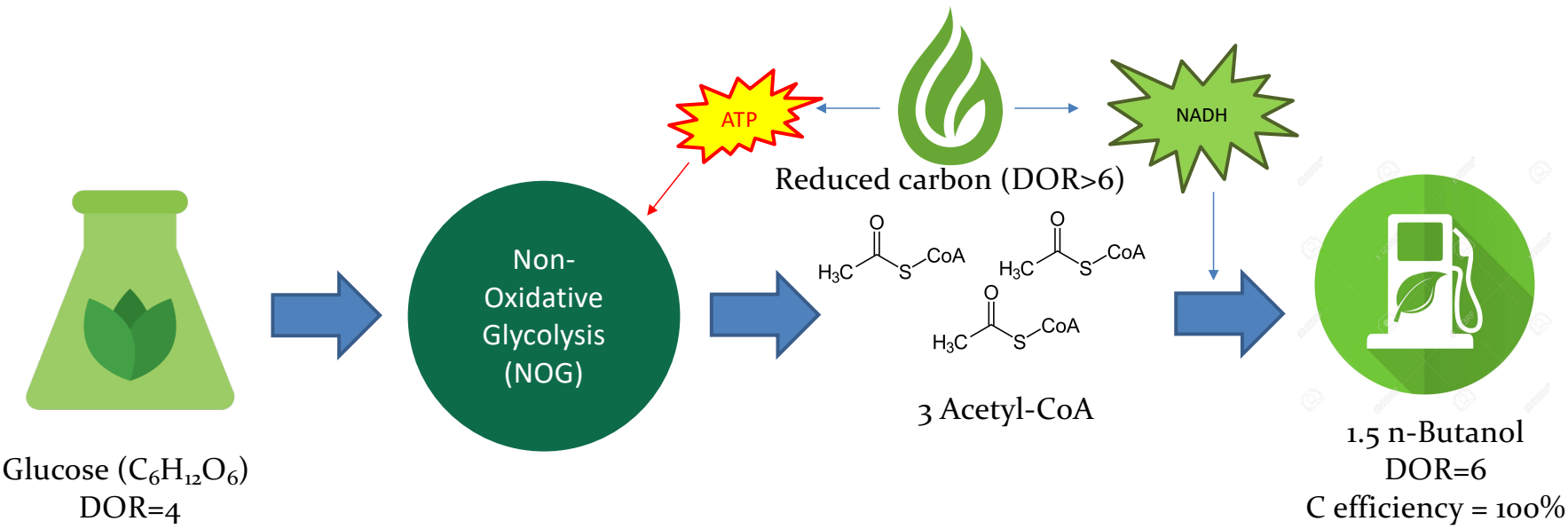
Production of reducing equivalents (and ATP) from "reduced-carbon" energy sources



Methane reforming/"bioreforming"

$$CH_4 + H_2O \longrightarrow CH_2O + 2H_2 \qquad \Delta G° = +184.2\ kJ/mol$$

$$CH_4 + 2NAD^+ + H_2O \longrightarrow CH_2O + 2NADH + 2H^+ \qquad \Delta G° = +115.1\ kJ/mol$$

ATP

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Methane as a "reduced-carbon" energy source

$$\text{Methane } (CH_4) + CO_2 + CoA \longrightarrow \text{Acetyl-CoA} + H_2O$$

$$CH_4 + 2NAD^+ + H_2O \longrightarrow CH_2O + 2NADH + 2H^+$$

$\Delta G° = 41$ kJ/mol

$\Delta G° = +115.1$ kJ/mol

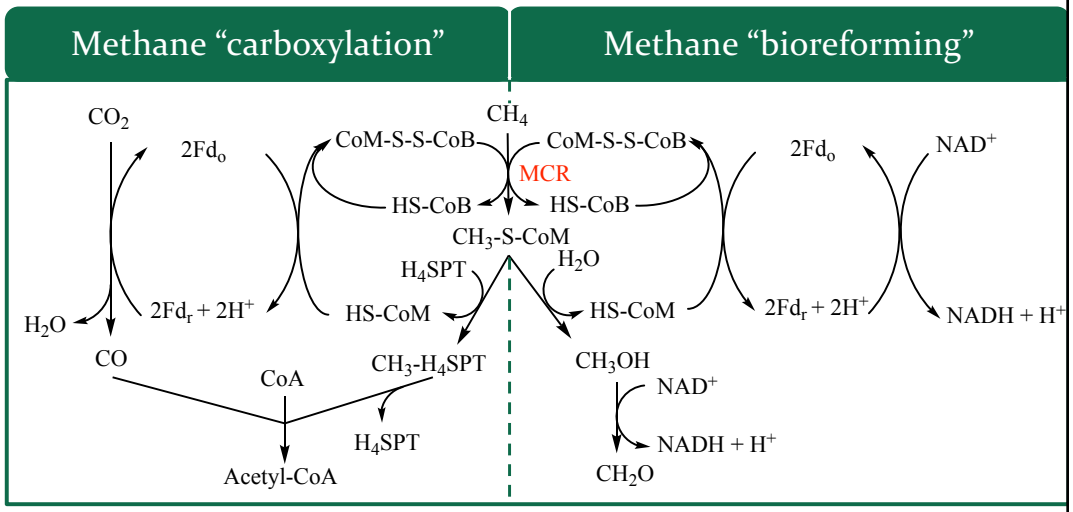
ATP

Activation method

- Shouldn't be MMO as it loses electrons to oxygen
  - Could recover electrons through water splitting but significant amount of energy is lost as waste heat
- Only known method is based on **MCR**

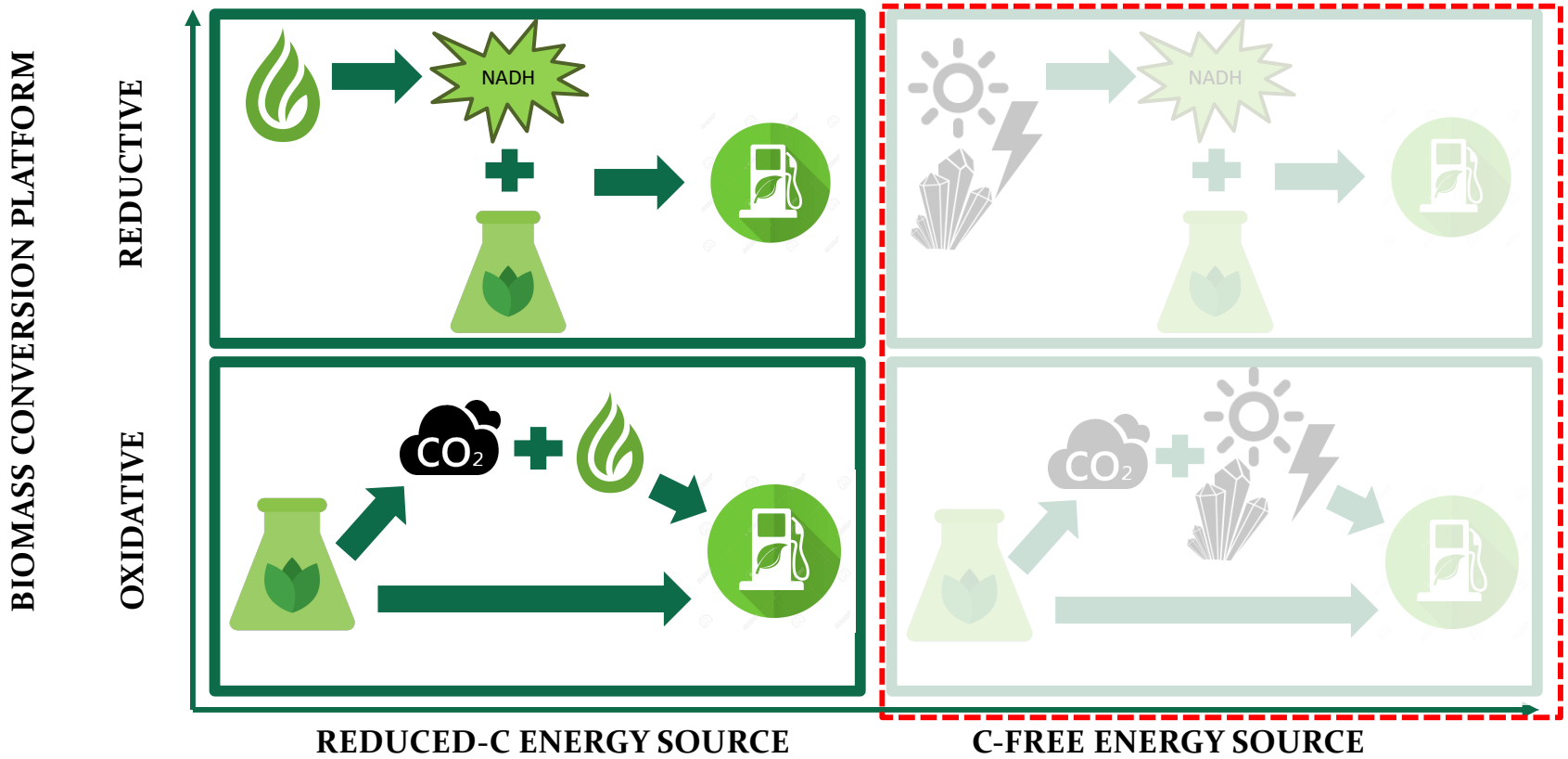
Metabolic pathways

- CO_2, O_2, metal ions, NOx, SOx, POx etc as terminal electron acceptors
- Existing pathway in nature vs synthetic pathway
- Thermodynamically uphill → Source of ATP?
- Implement in fast-growing industrial organism or methylotroph or cell-free system.



Can leverage <u>REMOTE</u> Program: Frontiers in microbiology 9, 1322, 2018; Nature communications 9, 1642, 2018; Front. Environ. Sci., 27 July 2018

What about using "C-free" energy



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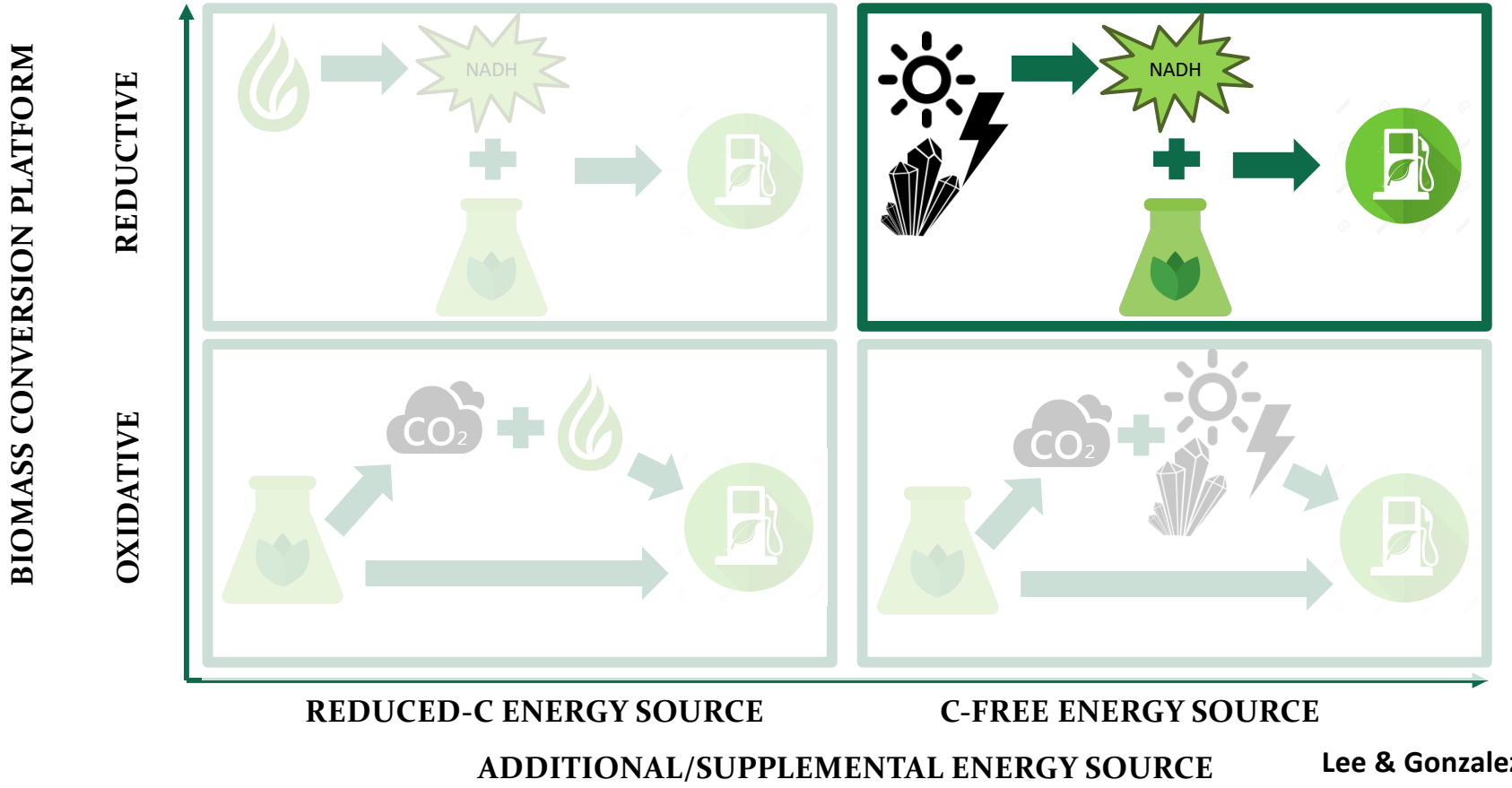
"Non-oxidative" conversion platform & "C-free" energy



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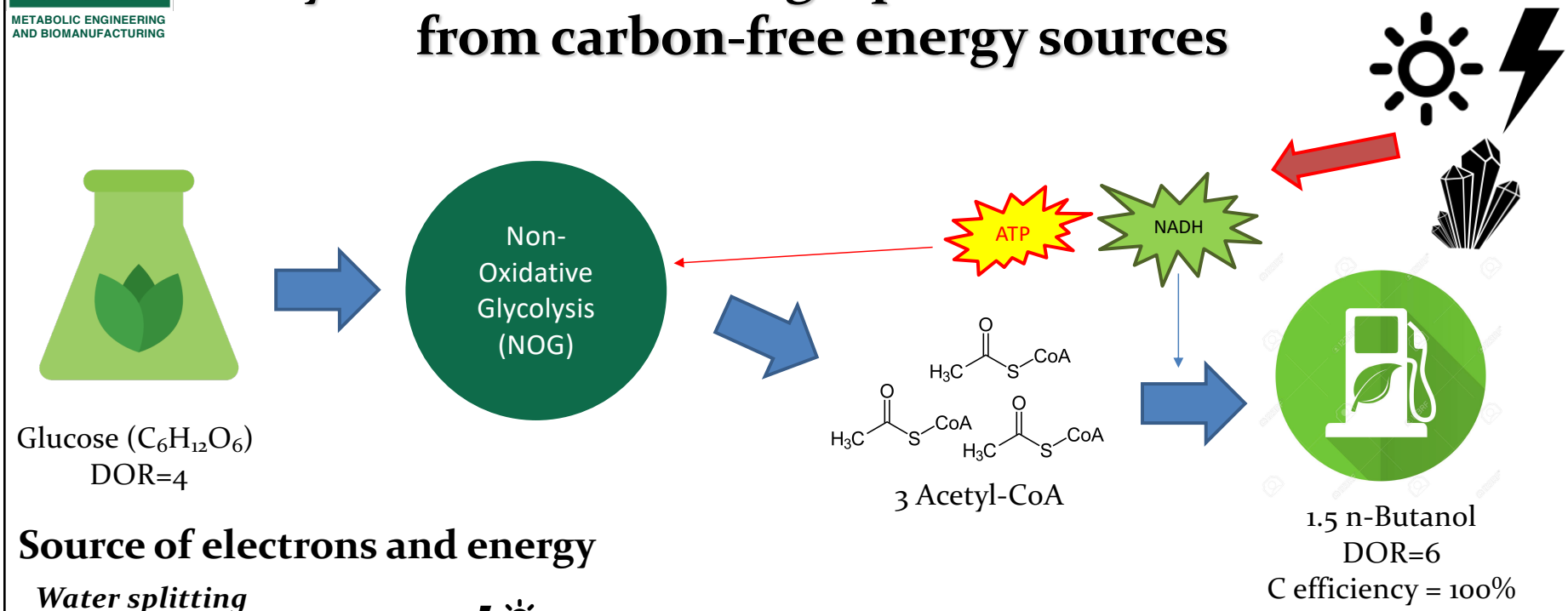
Synthesis of reducing equivalents and ATP from carbon-free energy sources



Source of electrons and energy

Water splitting

$$\text{Glucose } (C_6H_{12}O_6) + 1.5\ H_2O \xrightarrow{\text{light}} 1.5 \text{ Butanol } (C_4H_{10}O) + 3O_2 \qquad \Delta G° = 1066 \text{ kJ/mol}$$

Minerals

$$\text{Glucose } (C_6H_{12}O_6) + 2S + 3.5H_2O \xrightarrow{\text{minerals}} 1.5 \text{ Butanol } (C_4H_{10}O) + 2SO_4^{2-} + 4H^+ \qquad \Delta G° = -163.0 \text{ kJ/mol}$$

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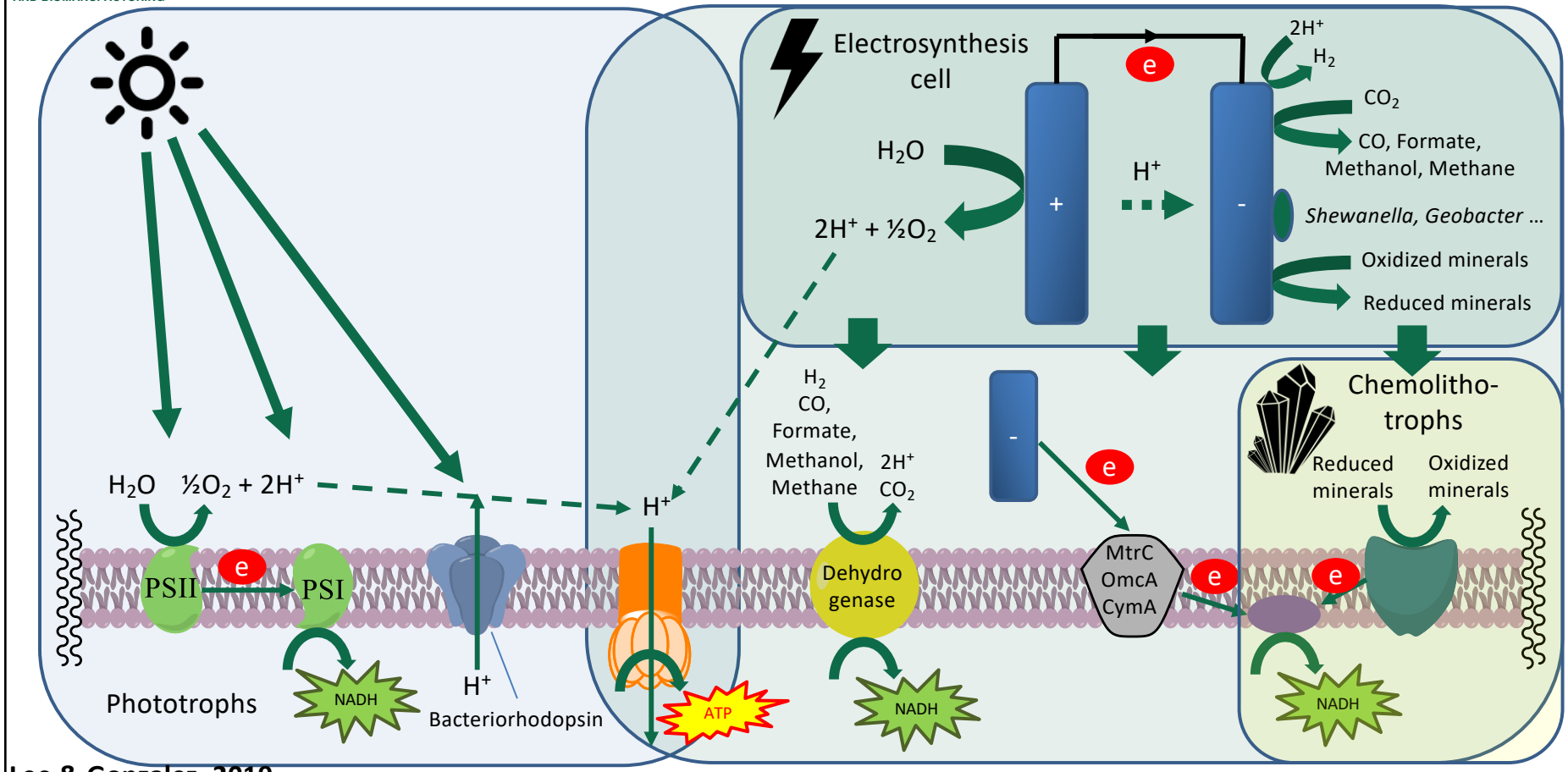
Reducing equivalents & ATP from C-free energy



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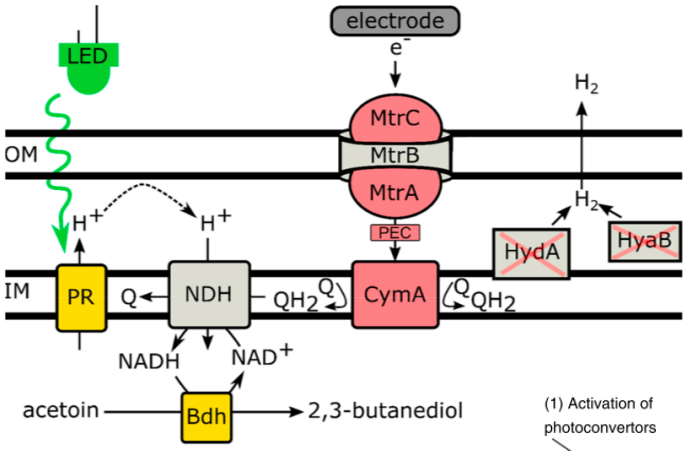
Reducing equivalents & ATP from C-free energy

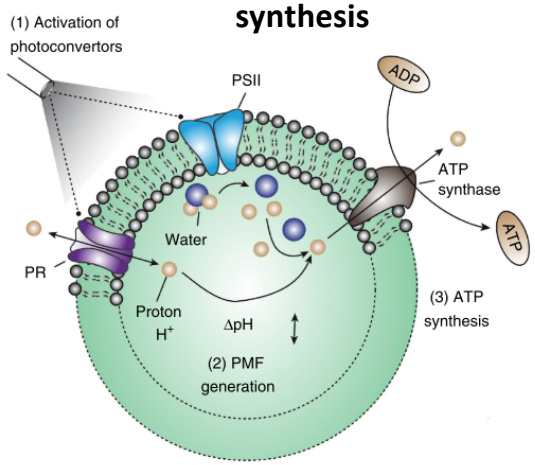
METABOLIC ENGINEERING AND BIOMANUFACTURING

Reversing EET in Shewanella**



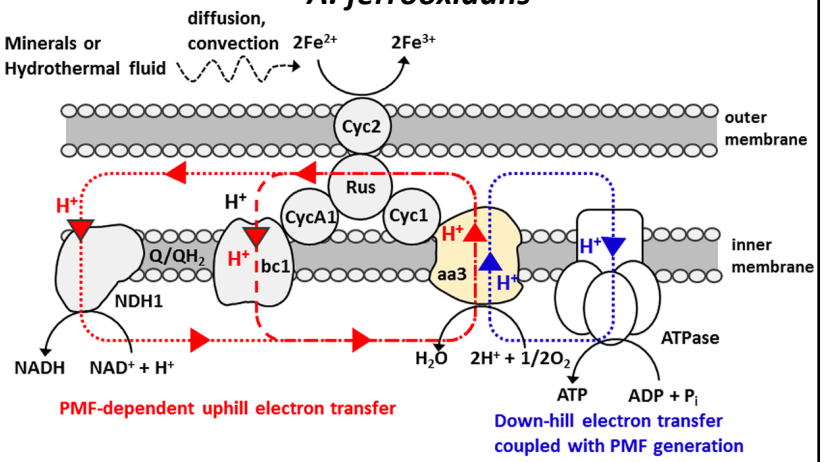
ACS Synth. Biol. 2019, 8, 1590–1600

In vitro ATP synthesis



Nat Biotechnol. 2018 Jul;36(6):530-535

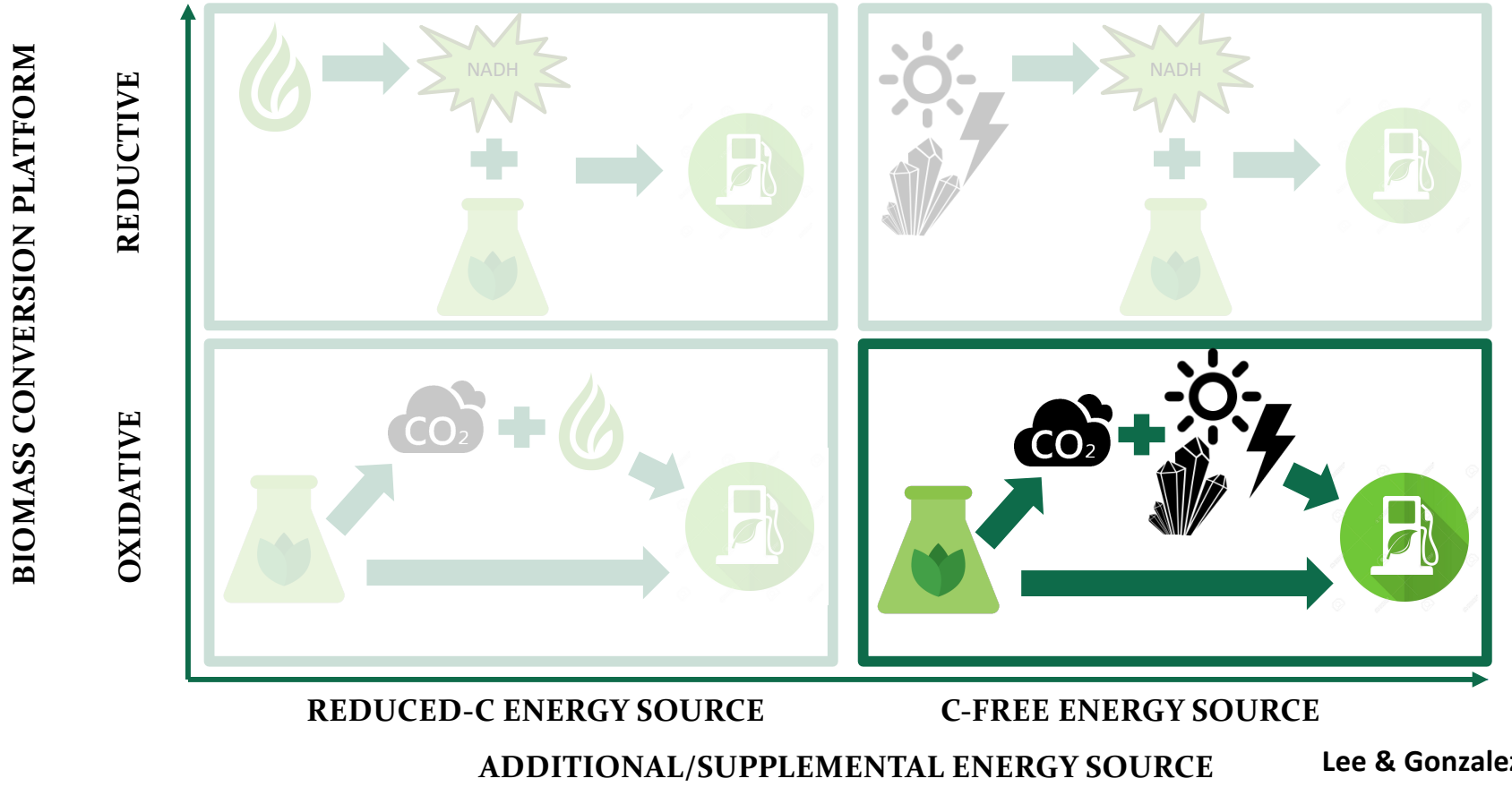
NADH and ATP from minerals**
A. ferrooxidans



Front Microbiol. 2015, 6:994.

**Can leverage ELECTROFUELS Program

“Oxidative” conversion platform & “C-free” energy



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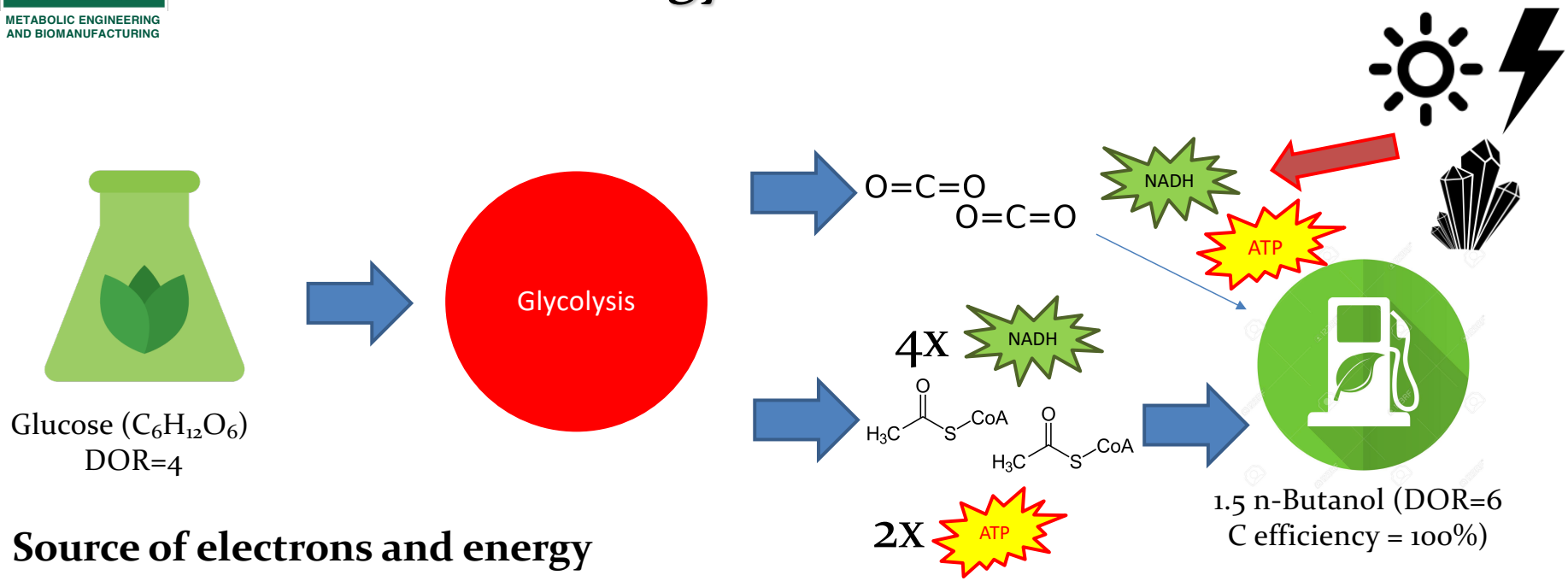
"Carbon-free" energy sources for CO_2 fixation



Source of electrons and energy

Water splitting

$$2CO_2 + 2.5H_2O \xrightarrow{\text{light}} 0.5\ \text{Butanol}\ (C_4H_{10}O) + 3O_2 \qquad \Delta G° = +1332.3\ \text{kJ/mol}$$

Minerals

$$2CO_2 + 2S + 4.5H_2O \xrightarrow{\text{minerals}} 0.5\ \text{Butanol}\ (C_4H_{10}O) + 2SO_4^{2-} + 4H^+ \qquad \Delta G° = +102.8\ \text{kJ/mol}$$

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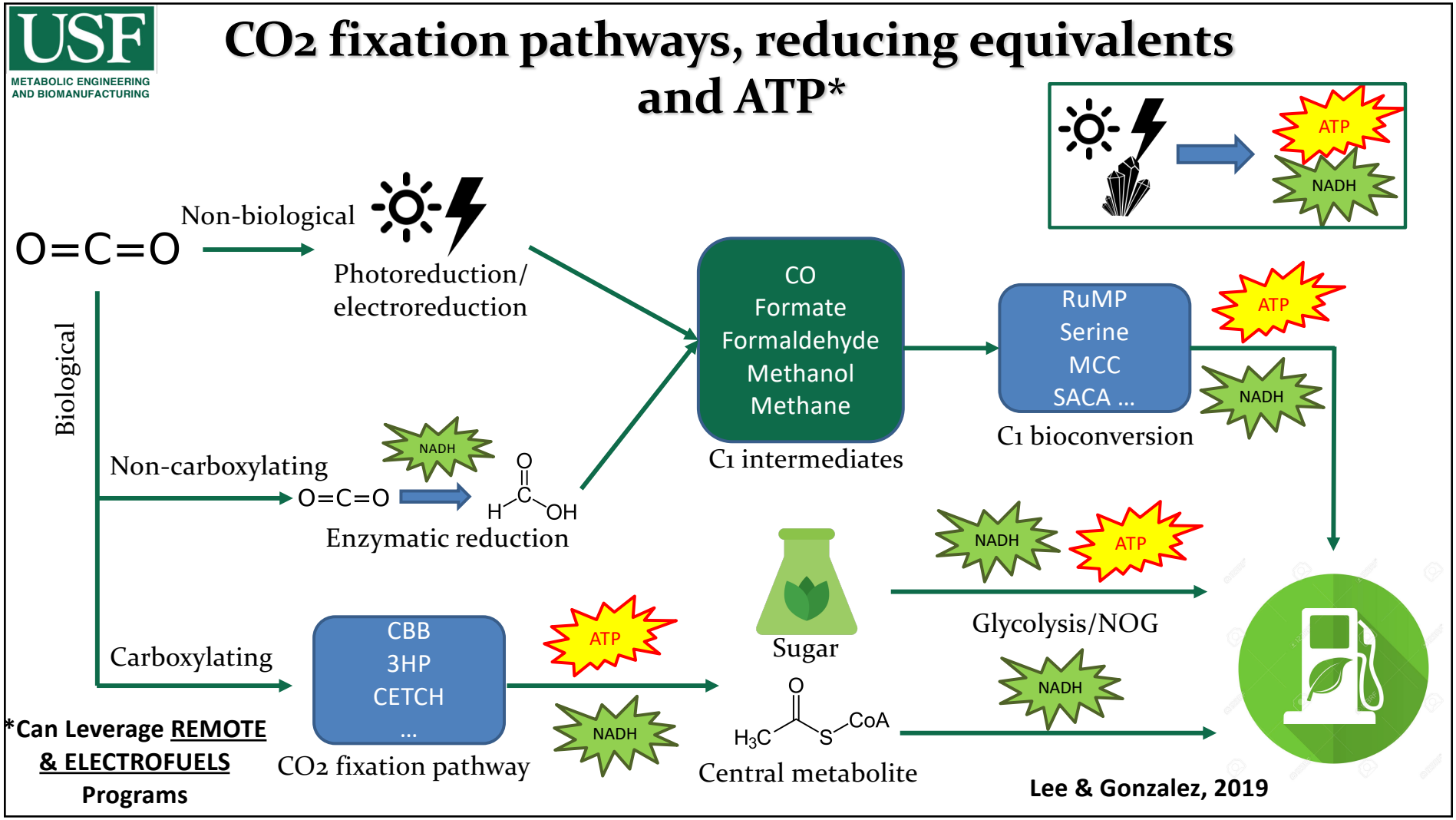
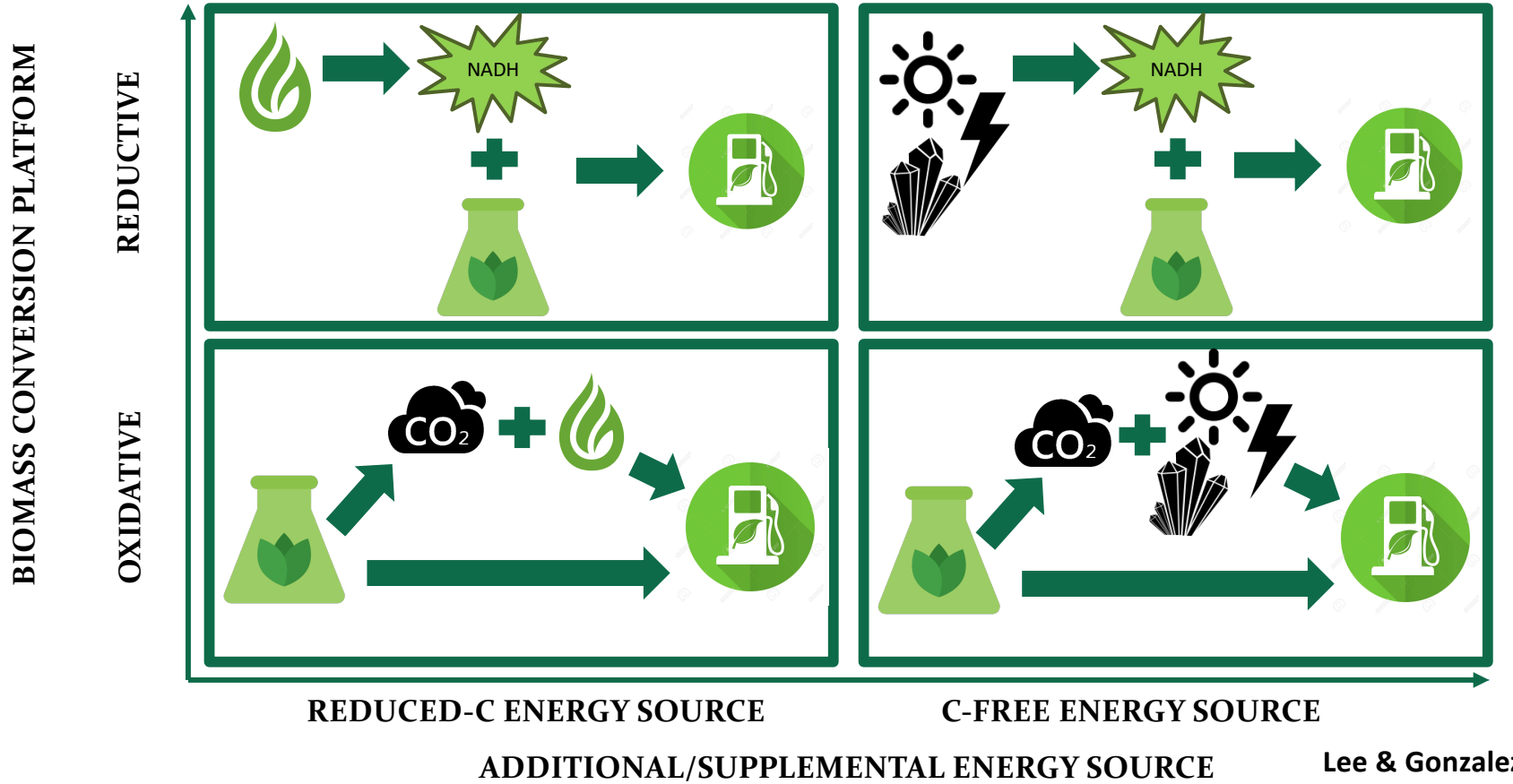
CO_2 fixation pathways, reducing equivalents and ATP*



Summary



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Summary

BIOMASS CONVERSION PLATFORM	REDUCED-C ENERGY SOURCE	C-FREE ENERGY SOURCE
REDUCTIVE	• Efficient activation of reduced substrate • Efficient conversion of activated molecule to reducing equivalents & ATP • Carbon-conserving metabolic pathway • Inorganic electron acceptors	• Efficient conversion of solar, electricity and minerals to NADH & ATP • Effective electron transfer from cathode to electron shuttles/microbes • Carbon-conserving metabolic pathway • Photo-/chemo-/electro-heterotrophs
OXIDATIVE	• Efficient activation of reduced substrate • Efficient conversion of activated molecule utilizing CO_2 as electron acceptor • CO_2 fixation (carboxylating & non-carboxylating) • Inorganic electron acceptors	• Efficient conversion of solar, electricity and minerals to NADH & ATP • Effective electron transfer from cathode to electron shuttles/microbes • CO_2 fixation (carboxylating & non-carboxylating) • Efficient CO_2 fixation pathways • Photo-/chemo-/electro-autotrophs

ADDITIONAL/SUPPLEMENTAL ENERGY SOURCE

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Summary

BIOMASS CONVERSION PLATFORM

REDUCTIVE

OXIDATIVE

- "Biological energy" (NAD(P)H & ATP) from solar, electricity, minerals, others?
- Enzymes & pathways
  - C1 bioconversion
  - Carbon conserving pathways
  - Activation of C-H bonds (and conversion of activated molecule)
- Hosts & systems:
  - Model/industrial/novel microbes: Photo-/chemo-/electro-autotrophs/-heterotrophs
  - Single cultures, co-cultures/consortia
  - *In vitro* systems
- Processes: single step/one-pot *vs* multi-stage; all bio *vs* chem-bio; mass & heat transfer; process intensification
- Tools: synthesis (genetic tools), analysis (molecular and system modeling)

REDUCED-C ENERGY SOURCE

C-FREE ENERGY SOURCE

ADDITIONAL/SUPPLEMENTAL ENERGY SOURCE

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