**Conversion of *Escherichia coli* to Generate Required Biomass from Methane**
(Technical Paper)

**Battle of the Bovines: How Differences Between Plains Indigenous Nations and American Cultures Affect Preferred Bovine Protein Choice**
(STS Paper)

A Thesis Prospectus
In STS 4500
Presented to
The Faculty of the
School of Engineering and Applied Science
University of Virginia
In Partial Fulfillment of the Requirements for the Degree
Bachelor of Science in Biomedical Engineering

**Caroline Nozomi Davis**
Fall 2022

Technical Team Members:

Austin Amacher

Gina Brown

On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

ADVISORS

George Prpich, PhD, Department of Chemical Engineering

Bryn E. Seabrook, PhD, Department of Engineering and Society

## Introduction

Anthropogenic methane emissions are one of the primary agents in the rapidly destabilizing environment. In the atmosphere, methane is 80 times more effective at trapping heat than carbon dioxide. As such, methane accounts for about 30 percent of global warming since the beginning of the industrial period. One of the greatest contributors to methane emissions is the global livestock industry. As global populations grow, so does the need for food, directly increasing methane emissions. Bovines, like cows, are the primary culprits of mass methane emissions in the livestock industry ("Control methane to slow global warming—fast", 2021). Land clearing for grazing, feed production, manure production, and rumination, the process of digesting feed by fermentation, are causes of methane emissions in livestock production (Kauffman et al., 2022).

While the livestock industry contributes to 14.2 percent of anthropogenic methane emissions, the cattle industry contributes 2 percent and the dairy industry contributes 4 percent in the US (Quinton, 2019). As such, strategies for reducing methane emissions must combat methane emissions in beef and dairy production. The United Nations Environment Programme cites the need for new agricultural technologies, plant-based diets, and alternative sources of protein in order to reduce methane emissions ("Methane emissions are driving climate change. Here's how to reduce them", 2021). However, the common-sense approach of reducing beef and dairy consumption to reduce methane emissions clashes with society's desire for these products. As more people shift from lower to middle classes, meat consumption rises ("Control methane to slow global warming—fast", 2021). Thus, the reduction of methane emissions in cattle production requires innovative solutions that respect the globally increasing desire for beef and dairy products.

I will discuss two strategies for reducing methane emissions in the bovine industry. One strategy is to engineer novel metabolic behaviors in microorganisms like bacteria to convert methane to organic matter in a technical project. Bacterial engineering requires technical knowledge of genetic engineering and mechanisms of metabolic pathways. The second approach is to explore alternative bovine products that produce less methane than cattle production in a research paper. The research paper will examine bison, a native bovine species of North America. I will examine the differences of Plains Indigenous Nations and American cultures that impacts bison husbandry in America. By equally combating excessive cattle meat consumption with engineered microorganisms and promoting bison meat as a better bovine protein product, the American bovine industry will reduce methane emissions while still supplying demanded products to consumers.

**Technical Topic**

One of the grand challenges in sustainable synthetic biology is to engineer bacteria like *Escherichia coli* to convert greenhouse gases into organic matter (Erb et al., 2019). One such unsolved problem is engineering *E. coli* to consume methane to generate all required biomass, or all required energy for an organism. Recent work in genetically modifying *E. coli* to consume $CO_2$ for all required biomass has been successful. Researchers implemented the Calvin-Benson-Bassham cycle, a $CO_2$-fixing cycle found in photosynthesis, into *E. coli* (Gleizer et al., 2019).

However, the $CO_2$-fixing strategy cannot be applied to methane emissions because the Calvin-Benson-Bassham cycle does not break down methane (Khmelenina et al., 2018). Instead my technical capstone team will examine a novel approach to engineer *E. coli*. We will engineer an *E. coli* strain to break down atmospheric methane into $CO_2$, formate, and formaldehyde by a non-native protein, methane monocygenase, and utilize the $CO_2$ product in a non-native Calvin-Benson-Bassham cycle to synthesize all required biomass from methane (Figure 1). The proposed method of methane fixation is resolvable in three aims.

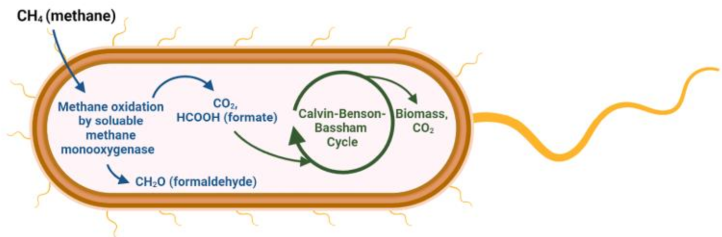


**Figure 1. Schematic representation of Engineered *E. coli* (Davis, 2022).**
Engineered *E. coli* oxidizes methane with non-native methane monooxygenase to produce $CO_2$, formate, and formaldehyde (blue). Oxidized $CO_2$ (carbon source) and formate (electron donor) are used in the non-native $CO_2$-fixing Calvin Benson Bassham cycle to produce biomass and $CO_2$ as a byproduct (green).

To begin, the utilization of a published genome library like EcoCyc in random forest regression modeling, which is a machine learning algorithm that finds correlations between variables, will identify which genomic sequences direct native metabolic behaviors in *E. coli* (Keseler et al., 2021). Then, a flux balance, which is a mathematical model of a metabolic network, will further identify genomic perturbations of genetic deletions that will remove the native metabolic behaviors in *E. coli* (Antonovsky et al., 2016). Further, the insertion of a non-native genetic sequence from a methanotrophic, or methane-consuming, bacteria will allow for *E. coli* to synthesize methane monooxygenase (sMMO), a protein that fixes methane into $CO_2$, formate, and formaldehyde (Khmelenina et al., 2018). The second insertion of an encoding sequence for the photosynthesis-derived Calvin-Benson-Bassham $CO_2$-fixing cycle will allow *E. coli* to produce the required biomass from methane-fixed $CO_2$. Finally, the application of selective conditions for the rewired metabolic configurations through a low-glucose, high-methane environment will allow for the desired metabolic behavior (methane fixation) to be linked to the fitness, or survival of the *E. coli* strain. Implementation of methane-fixing behaviors in *E. coli* will reduce the amount of methane released into the atmosphere (Gleizer et al., 2019).

If engineered *E. coli* are able to utilize methane as a primary carbon source, there are two potential impacts. The first impact is the improvement of global synthetic biology knowledge due to the demonstrated techniques in the technical project (Erb et al., 2019). A second impact is the reduction of methane emissions in the agriculture industry both domestic and global ("Control methane to slow global warming—fast", 2021). Global agriculture industries will be able to remediate methane emission epicenters like manure fields by applying a colony of this strain to the environment. Further implementation of the safe engineered *E. coli* in the bovine's digestive tract will reduce methane emissions from rumination.

**STS Topic**

Before colonizers stole Turtle Island and what is currently the United States and Canada from Indigenous Nations, many Nations relied on bison for textiles, food, infrastructure, and identity. For example, the Siksikaitsitapi Confederacy (Blackfoot Confederacy), consisting of the Siksika Nation (Blackfoot), Kainai Nation (Blood Tribe), Piikani Nation (Peigan), and Amskapi Piikuni (Blackfeet) Nation, centered bison in their cultural identities (Blackfoot Confederacy Tribal Council, 2021). Manifest Destiny, the "claim" to Western lands in the United States by white settlers, clashed with the Native Nations' cultures of bison hunting in the Great Plains. Treaties restricted Native Nations to borders, dismantling Native life alongside the migrating bison. The US government instantiation programs with the US Army and private organizations to exterminate the bison, in order to "kill the Indian" (Phippen, 2016).

However, bison are considered a more sustainable protein option than cattle. While bison belong to the bovine family and thus produce methane during digestion, rumination, bison are a keystone species of the Great Plains, which means they have a significant impact on the environment even in small populations. Bison improve Plains biodiversity through choice of forage and roaming behaviors. Cattle, on the other hand, are not considered keystone species, but rather a non-native species on the Plains (Kohl et al., 2013).

Further, bison meat is healthier than beef because bison contains a lower total fat content compared to beef. Additionally, bison meat contains a higher ratio of polyunsaturated fatty acids (PUFA), which are essential fatty acids for neural development and cellular health, than saturated fatty acids (SFA), which are nonessential fatty acids that increase risk of cardiovascular disease ("Types of Fat", Harvard T.H. Chan School of Public Health). Despite evidence supporting the bison as a sustainable, healthy choice to satisfy protein consumption, beef remains the primary red meat of choice in the United States (McDaniel et al, 2018).

Thus, it is crucial to discuss the societal differences between Plains Indigenous Nations and American culture that cause differing preferences of bovine meat products. There are multiple stakeholders in the conversation, including individual Indigenous Nations on the Plains that support bison husbandry, the cattle industry, the US government, and the US general public. Each individual stakeholder supports either bison or beef as an artifact of protein source in their diet.

Additionally, the support of either bison or beef as preferred artifacts are based on the collective views of the stakeholders. Thus, I will utilize the concept of the social construction of technology (SCOT) to discuss the societal differences between Plains Indigenous Nations and American consumer culture that results in the differing bovine choice. SCOT consists of four elements. The first element is interpretive flexibility, which defines technology, or a given artifact, design as a multi-dimensional process whose outcomes depend on social circumstances. The second element is the impact of the relevant social group on an artifact's interpretation. Closure and stabilization compose the third element of SCOT, defined as the conclusion of a technology's design only after conflicting ideas of an artifact are resolved to every impacted social group. The final element is the wider sociocultural context that is defined by the artifact's development (Klein and Kleinman, 2002).

While SCOT is a strong toolkit to describe the differences in Plains Indigenous Nation and American culture, there are some criticisms that reduce the dimensionality of the SCOT framework. One such critique is that SCOT places too much emphasis on agency and neglects pre-existing structures that play a role in an artifact's development. For example, a comparison of bison versus cattle may focus too much on the cultural impacts of each protein source and neglect the overwhelming amount of cattle-focused agricultural structures that overwhelmed the Great Plains from colonialism. Another critique is the division of society into groups, which is often seen as a pluralistic perspective. SCOT defines groups as equally represented in an artifact's development, which ignores disproportionate power structures like the power structure between the American government and Indigenous peoples (Klein and Kleinman, 2002). The destruction of Indigenous pedagogies by American ideals will be transposed to the current cattle-heavy agricultural sector to combat this critique.

Indigenous voices are silenced through purposeful erasure by the American government as aforementioned through the decline of bison populations in the Great Plains. The Indigenous methods of bison husbandry are rarely discussed in American society, but rather are constrained to Indigenous pedagogies. Thus, it is critical to examine the social impacts of bison on Indigenous Nations compared to the social impacts of cattle with the SCOT framework.

**Research Question and Methods**

Research Question: How do differences between Plains Indigenous Nations and American cultures affect preferred bovine protein choice?

As aforementioned, exploring cultural differences between Plains Indigenous Nations and American culture will highlight the discrepancies between bovine products in the US diet. To pursue this exploration, I will analyze multiple types of sources with documentary research methods, which is the process of collecting peer-reviewed sources and exploring the significance of each source. Academic Journal articles from peer-reviewed journals like *Nature* and *Nutrition Research*, government reports from the US Department of Agriculture, news articles from *The Atlantic*, and editorials from scientific journals like *Nature* and global organizations like the United Nations will be employed to discuss the differences between Plains Indigenous Nations and American culture. It is important to note that Indigenous voices will be identified by their tribal citizenship. Certain keywords like “Plains Indigenous bison culture,” “bovine comparisons,” “American cattle industry,” and “bison history” are used to identify the significance of bison to Plains Indigenous Nations and the beef-heavy American diet. I will organize findings in a chronological manner, from Turtle Island’s pre-colonialism era to the decline of the bison to the rise of cattle and finally to current bovine methodologies and Indigenous futurisms. Diverse literature reviews will enunciate the multiple stakeholders and cultural impacts of bison and cattle to each stakeholder.

## Conclusion

To summarize, there are two deliverables in this project. The first deliverable is a technical project. The expected outcome of the technical portion is an engineered *E. coli* strain that successfully consumes methane as a primary carbon source. As a result, multiple industries will have access to an additional tool for methane emissions reduction. Further, the second deliverable is an STS research paper on the impact of Plains Indigenous versus American culture on preferred bovine protein consumption. The STS paper is supported by the social construction of technology (SCOT) framework. The STS deliverable will promote the implementation of more bison meat in the American cattle industry, supported by Indigenous bison husbandry. Overall, both deliverables attempt to combat anthropogenic, or human-caused, methane emissions from a technological and sociocultural perspective.

## References

Antonovsky, N., Gleizer, S., Noor, E., Zohar, Y., Herz, E., Barenholz, U., Zelcbuch, L., Amram, S., Wides, A., Tepper, N., Davidi, D., Bar-On, Y., Bareia, T., Wernick, D. G., Shani, I., Malitsky, S., Jona, G., Bar-Even, A., & Milo, R. (2016). Sugar synthesis from co2 in escherichia coli. *Cell*, *166*(1), 115–125. https://doi.org/10.1016/j.cell.2016.05.064

Control methane to slow global warming—Fast. (2021). *Nature*, *596*(7873), 461–461. https://doi.org/10.1038/d41586-021-02287-y

*Cows and climate change*. (2019, June 27). UC Davis. https://www.ucdavis.edu/food/news/making-cattle-more-sustainable

Erb, T. J., Keller, P., & Vorholt, J. A. (2019). Escherichia coli in auto(trophic) mode. *Cell*, *179*(6), 1244–1245.

Gleizer, S., Ben-Nissan, R., Bar-On, Y. M., Antonovsky, N., Noor, E., Zohar, Y., Jona, G., Krieger, E., Shamshoum, M., Bar-Even, A., & Milo, R. (2019). Conversion of escherichia coli to generate all biomass carbon from co2. *Cell*, *179*(6), 1255-1263.e12. https://doi.org/10.1016/j.cell.2019.11.009

Kauffman, J. B., Beschta, R. L., Lacy, P. M., & Liverman, M. (2022). Livestock use on public lands in the western USA exacerbates climate change: Implications for climate change mitigation and adaptation. *Enviornmental Management*, *69*(6), 1137–1152. https://doi.org/10.1007/s00267-022-01633-8

Keseler, I. M., Gama-Castro, S., Mackie, A., Billington, R., Bonavides-Martínez, C., Caspi, R., Kothari, A., Krummenacker, M., Midford, P. E., Muñiz-Rascado, L., Ong, W. K., Paley, S., Santos-Zavaleta, A., Subhraveti, P., Tierrafría, V. H., Wolfe, A. J., Collado-Vides, J., Paulsen, I. T., & Karp, P. D. (2021). The ecocyc database in 2021. *Frontiers in Microbiology*, *12*, 711077. https://doi.org/10.3389/fmicb.2021.711077

Khmelenina, V. N., Colin Murrell, J., Smith, T. J., & Trotsenko, Y. A. (2018). Physiology and biochemistry of the aerobic methanotrophs. In F. Rojo (Ed.), *Aerobic Utilization of Hydrocarbons, Oils and Lipids* (pp. 1–25). Springer International Publishing. https://doi.org/10.1007/978-3-319-39782-5_4-1

Klein, H. K., & Kleinman, D. L. (2002). The social construction of technology: Structural considerations. *Science, Technology, & Human Values*, *27*(1), 28–52. https://doi.org/10.1177/016224390202700102

Kohl, M. T., Krausman, P. R., Kunkel, K., & Williams, D. M. (2013). Bison versus cattle: Are they ecologically synonymous? *Rangeland Ecology & Management*, *66*(6), 721–731. https://doi.org/10.2111/REM-D-12-00113.1

McDaniel, J., Askew, W., Bennett, D., Mihalopoulos, J., Anatharaman, S., Fjeldstad, A., Rule, D. C., Nanjee, N. M., Harris, R., & Richardson, R. S. (2013). Bison meat has a lower atherogenic risk than beef in healthy men. *Nutrition Research*, *33*(4), 293–302.

*Methane emissions are driving climate change. Here's how to reduce them.* (2021, August 20). UNEP. http://www.unep.org/news-and-stories/story/methane-emissions-are-driving-climate-change-heres-how-reduce-them

Phippen, J. W. (2016, May 13). Kill every buffalo you can! Every buffalo dead is an Indian gone. *The Atlantic*. https://www.theatlantic.com/national/archive/2016/05/the-buffalo-killers/482349/

*Siksikaitsitapi – Blackfoot Confederacy tribal council*. (n.d.). Retrieved October 27, 2022, from https://blackfootconfederacy.ca/

*Types of fat*. (2014, June 9). The Nutrition Source, Harvard T. H. Chan School of Public Health. https://www.hsph.harvard.edu/nutritionsource/what-should-you-eat/fats-and-cholesterol/types-of-fat/