Leveraging Enzyme Excretion Systems for the Cell-Free Synthesis of Lactic Acid;

Corporate Producer Dominance in the FPCN: How a Moral Shift Led to the Modern American Health Crisis

A Thesis Prospectus

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Introduction

How often do you think about the chemicals you put in your body? Probably not as often as you should, considering more than 50% of Americans do not read the ingredients listed on their food (Goyal & Deshmukh, 2018, p. 56). Food preservatives, an ingredient used to prolong the shelf life of many popular foods and prevent food-borne diseases (Pisoschi et al., 2018, p. 923), for example of a slew of worrisome side effects associated with their consumption (Manuela Silva & Cebola Lidon, 2016, p. 371). This research will examine how a moral shift has ceded control within the food production-consumption network (FPCN) to corporate producers leading to the American health decline. A three step solution will be proposed to counteract the power imbalance and restore consumer autonomy within the FPCN. Lactic acid is a valuable biocommodity that produced approximately eight billion dollars in revenue as an antioxidizing agent in 2005 (Datta & Henry, 2006, p. 1123). In order to understand the apathetic culture surrounding American food consumption, this research will use Moral Foundations Theory to investigate the various interests influencing the consumption of food ingredients and how social networks are being manipulated to both alter and maintain the corporate producer power within the FPCN. A current resistance to the status quo will be analyzed and the effectiveness of the movement will be determined based on their network capabilities (Lockie, 2002, p. 289). Without this analysis, many current health crises will continue and no effective solutions will be proposed. This paper will propose that the most effective solution is to have a moral retrograde, educate the public in nutritional matters, and use collective action to regain power for the consumer.

Technical Topic

Many industries are dependent on large quantities of biocommodities to continuously run their biochemical processes. Biocommodities, the cheap raw materials essential for almost every chemical and biochemical process, are inexpensive compared to high value products. The cost is heavily reliant on the feedstock cost which accounts for 30%-70% of production expenses (Zhang, 2010, p. 669). One of the most versatile biocommodities in the current market is lactic acid which has applications in the pharmaceutical, cosmetic, food and beverage, and biodegradable plastics industries (Datta & Henry, 2006, pp. 1120-1123). All of these industries are vital to standard products in American life. This already sophisticated market is expected to grow at a rapid rate. Lactic Acid production was projected as a 3.5 billion dollar industry in 2022 and is projected to double by 2031 allowing for a well-designed, cheap production process to crack into the market (Datta et al., 1995, p. 225).

One of the cutting edge methods to cheaply produce biocommodities is cell free biocatalysis. Cell-free biotransformations (SyPaB) is the use of several enzymes to create catalytic networks outside of microbial organisms for the production of biochemical products. SyPaB was shown to increase product yield, improve process flexibility, and hasten reaction rate which will decrease the time required to produce commodities like lactic acid (Zhang, 2010, pp. 665-666). These enzymes are also recyclable (Wee & Ryu, 2009, pp. 4265-4267) without the downside of cell glucose consumption. Results from anaerobic cell catalysis experimentation find that 10% of the feedstock is lost from the feed stream (Zhang, 2010, p. 669) with more unconverted feedstock being consumed in recycle streams. By removing cell consumption of feedstock in both the initial and recycled streams, the cost is decreased as the efficiency increases making cell free catalysis a viable alternative to cell fermentation. Cell free biotransformation along SyPaB also decreases the amount of waste products because other enzymes within the cell can be removed before reactions are performed if enzyme selection is effectively performed (Zhang, 2010, pp. 665-666).

The production of lactic acid still faces several constraints, chief among them is waste production (Alves de Oliveira et al., 2018, pp. 223-224). Cell free biotransformations also have less data than traditional cell fermentations (Zhang, 2010, p. 664). Additional data may need to be gathered in this project in order to develop an efficient process that mitigates the side products and waste accumulating in lactic acid production. Waste production is accompanied by environmental restrictions that severely reduce allowable production and increase the cost compared to less sustainable and traditional alternatives (Alves de Oliveira et al., 2018, p. 220). Cell free biotransformations should reduce these lactic acid side product concerns, but complete elimination is not a reasonable expectation. The aim of this project is the cell-free production of lactic acid because we want to reduce the cost of pure lactic acid mass manufacture so that we reduce the price of various medical, food, and plastic alternative products that consumers use daily.

Various unit operations will be employed to create lactic acid from a cell-free reactor. First, we will ferment bacillus subtilis in a retentostat using LB broth as a growth media (Cruz Ramos et al., 2000). This microbe is capable of secreting enzymes which will help increase the purity of the system from the beginning (Abedi & Hashemi, 2020). However, the specifics of the genetic engineering required to produce such a cell line are out of the scope of this project. Next, a disk stack centrifuge will be used to remove any cellular debris and to separate the cells from the secreted enzymes (Phanthumchinda et al., 2018). The supernatant containing our target enzymes and other small secreted molecules will then undergo ultrafiltration to isolate our target enzymes (Sullivan et al., 2016). The enzymes of interest are GDH, KDGA, ALDH, DHAD, and L-LDH. The purified target proteins will be transferred to a holding tank until they are needed for the reactor, concluding the batch portion of the process. Enzymes and glucose from food waste will be fed into a continuous stirred tank reactor (CSTR) where the cell-free synthesis of lactic acid will occur (Hodgman & Jewett, 2012). The output will be various small molecule intermediates mixed in with the lactic acid product which will then be purified via nanofiltration, with enzymes being recycled back into the reactor (Phanthumchinda et al., 2018). The small molecules and lactic acid remaining will undergo sedimentation and liquid-liquid extraction with octanol and trioctylamine as solvents (Kumar & Thakur, 2019). The solvents will be removed using a distillation cascade and recycled into the downstream filtration process. The remaining output stream will be purified lactic acid which will then be packaged and sold in a liquid solution.

This project will be done by a group of four people (Gavin, Clare, Collin, and Ethan). The initial design will be created in the Fall semester for CHE 4474 and the project will be finished in the Spring semester for CHE 4476. The work will be split amongst the group as follows: Gavin and Clare will be designing the bioreactor and downstream processes needed to purify the lactic acid, Collin will be designing the lactic acid reactor used to produce the lactic acid from necessary enzymes, and Ethan will design the distillation cascade and conduct mathematical checks for the lactic acid production reactor. Ethan will also be researching and analyzing the economics behind the entire process. Every week, the team will meet up to discuss findings and report progress. All of the data needed for the material balances, operating conditions, and economic costs will be obtained from literature review. Aspen Plus V14 will be used to model the process and simulate its conditions. Matlab will be used to calculate individual material balance equations on each reactor.

STS Topic

According to the National Library of Medicine, 58% of Americans do not understand the food labeling meant to serve as a quick reflection of the material entering their bodies. 52% of Americans do not read the ingredients labels (Goyal & Deshmukh, 2018, p. 56). Lots of attention is given to other products such as phones and furniture, but scrutiny towards food additives is not culturally promoted to nearly the same level. Why do so many Americans neglect to examine the content of their food? This research seeks to understand why so many Americans are ignoring the content of their meals by examining the moral shift using Jonathan Haidt's Moral Foundations Theory and the effect it has had on health by investigating the agencies and interest groups who influence the consumer. By understanding why so many people are apathetic toward the food production-consumption network, this research will determine how changes to the current FPCN could improve public awareness of food content and improve the health of many Americans. Drawing upon structure from Stewart Lockie, this research will examine the willingness of Americans to cede authority to organizations to mediate contradictions and conflict (Lockie, 2002, p. 280) and how actors enroll the public into an authoritative network where decisions are neglected at the individual level (Lockie, 2002, pp. 281-284).

Food preservatives were going to be the focus of this analysis for three reasons. Lactic acid, the focus on the technical project, is a common food preservative without many of the side effects present in most other preservative options (Manuela Silva & Cebola Lidon, 2016, p. 371). This presents a pathway to improve public health by opting for lactic acid as an alternative to less healthy options which will serve as a baseline for explored solutions. The second reason is because there are notably dangerous side effects attributed to many commercially popular preservatives including allergen aggravation, introduction to carcinogens, and potential development of birth defects (Manuela Silva & Cebola Lidon, 2016, pp. 368-369). By examining why Americans ignore a food component with potentially dangerous consequences, the disregard surrounding unhealthy food components can be extrapolated. Finally, there are many interests surrounding food preservatives and a consumer backlash to preservatives that is beginning to gain strength (Lockie, 2002, pp. 279-282). With many competing and collaborating actors, the social network formed around food preservatives can serve as a good proxy for all food content apathy to be understood. For the sake of word count, the food preservative analysis was cut to examine the moral shift and the power dynamic within the network.

The lack of awareness by the consumer will be examined using Actor-Network Theory (ANT). Customer attention will be examined by investigating the relationships between consumers, retailers, manufacturers, and advertisers within the context of American food culture. This format of analysis is effective for examining consumer awareness because it sums the actions taken by many actors to form a cohesive network which allows the influence of multiple interests to be explored in relation to the consumer. ANT allows examination of the change each actor can have based on network limitations (Latour, 1999, pp. 17-18). This allows the change in consumer attention surrounding food preservatives to be investigated based on the food industry. Finally, ANT allows broader context to arise by considering the network (Latour, 1999, p. 18). By understanding the history of American food culture, the modern food production network and present consumer focus can be understood and altered in the interest of the consumer.

Conclusion

This project proposes a solution to the public health crises faced by the American population. As a result of the technical portion of this project, a cell-free chemical process will produce lactic acid from an input of glucose and cell-free enzymes aiming to reduce the cost of production. As a result of applying ANT to the apathetic American culture toward food preservatives, a better understanding of the various interests and the pressures they exert can be applied to understand that this apathy is broadly due to social pressures. By reducing the cost of lactic acid through the cell-free process, a food preservative lacking many of the side effects due to other preservatives would be produced. Due to the decreased cost, the lactic acid substitute should be embraced by manufacturers motivated by profits and the American apathy toward food content may be circumvented. Using this model as a guide, other dangerous food additives can be replaced if a suitable alternative is produced in a cost effective way.

References

Abedi, E., & Hashemi, S. M. B. (2020). Lactic acid production – producing microorganisms and substrates sources-state of art. *Heliyon, 6*(10), e04974. https://doi.org/10.1016/j.heliyon.2020.e04974

Alves de Oliveira, R., Komesu, A., Vaz Rossell, C. E., & Maciel Filho, R. (2018). Challenges and opportunities in lactic acid bioprocess design—From economic to production aspects. *Biochemical Engineering Journal, 133*(1), 219–239. https://doi.org/10.1016/j.bej.2018.03.003

Cruz Ramos, H., Hoffmann, T., Marino, M., Nedjari, H., Presecan-Siedel, E., Dreesen, O., Glaser, P., & Jahn, D. (2000). Fermentative Metabolism of Bacillus subtilis: Physiology and Regulation of Gene Expression. *Journal of Bacteriology, 182*(11), 3072–3080.

Datta, R., & Henry, M. (2006). Lactic acid: recent advances in products, processes and technologies — a review. *Journal of Chemical Technology & Biotechnology, 81*(7), 1119–1129. https://doi.org/10.1002/jctb.1486

Datta, R., Tsai, S.-P., Bonsignore, P., Moon, S.-H., & Frank, J. R. (1995). Technological and economic potential of poly(lactic acid) and lactic acid derivatives. *FEMS Microbiology Reviews, 16*(2-3), 221–231. https://doi.org/10.1111/j.1574-6976.1995.tb00168.x

Goyal, R., & Deshmukh, N. (2018). Food label reading: Read before you eat. Journal of Education and Health Promotion, 7(1), 56. https://doi.org/10.4103/jehp.jehp_35_17

Hodgman, C. E., & Jewett, M. C. (2012). Cell-free synthetic biology: Thinking outside the cell. *Metabolic Engineering, 14*(3), 261–269. https://doi.org/10.1016/j.ymben.2011.09.002

Kumar, A., & Thakur, A. (2019). Reactive extraction of lactic acid using environmentally benign green solvents and a synergistic mixture of extractants. *Scientia Iranica, 26*(6), 3456–3467. https://doi.org/10.24200/sci.2019.52233.2610

Latour, B. (1999). On Recalling ANT. *The Sociological Review, 47*(1), 15–25. https://doi.org/10.1111/j.1467-954x.1999.tb03480.x

Lockie, S. (2002). “The Invisible Mouth”: Mobilizing “the Consumer” in Food Production-Consumption Networks. *Sociologia Ruralis, 42*(4), 278–294. https://doi.org/10.1111/1467-9523.00217

Manuela Silva, M., & Cebola Lidon, F. (2016). Food preservatives - An overview on applications and side effects. *Emirates Journal of Food & Agriculture (EJFA), 28*(6), 366–373. https://doi.org/10.9755/ejfa.2016-04-351

Phanthumchinda, N., Thitiprasert, S., Tanasupawat, S., Assabumrungrat, S., & Thongchul, N. (2018). Process and cost modeling of lactic acid recovery from fermentation broths by membrane-based process. *Process Biochemistry, 68*, 205–213. https://doi.org/10.1016/j.procbio.2018.02.013

Pisoschi, A. M., Pop, A., Georgescu, C., Turcuş, V., Olah, N. K., & Mathe, E. (2018). An overview of natural antimicrobials role in food. European Journal of Medicinal Chemistry, 143, 922–935. https://doi.org/10.1016/j.ejmech.2017.11.095

Sullivan, C. J., Pendleton, E. D., Sasmor, H. H., Hicks, W. L., Farnum, J. B., Muto, M., Amendt, E. M., Schoborg, J. A., Martin, R. W., Clark, L. G., Anderson, M. J., Choudhury, A., Fior, R., Lo, Y.-H., Griffey, R. H., Chappell, S. A., Jewett, M. C., Mauro, V. P., & Dresios, J. (2016). A cell-free expression and purification process for rapid production of protein biologics. *Biotechnology Journal*, *11*(2), 238–248. https://doi.org/10.1002/biot.201500214

Wee, Y.-J., & Ryu, H.-W. (2009). Lactic acid production by Lactobacillus sp. RKY2 in a cell-recycle continuous fermentation using lignocellulosic hydrolyzates as inexpensive raw materials. *Bioresource Technology, 100*(18), 4262–4270. https://doi.org/10.1016/j.biortech.2009.03.074

Zhang, Y.-H. . P. (2010). Production of biocommodities and bioelectricity by cell-free synthetic enzymatic pathway biotransformations: Challenges and opportunities. *Biotechnology and Bioengineering*, *105*(4), n/a-n/a. https://doi.org/10.1002/bit.22630