

Arjun Bindra  
Jericho High School  
Jericho, New York, USA  
Tanzania, Sustainable Agriculture

## **Implementation of Microalgae-Bacteria Consortium Enriched Anaerobic Digestate In Tanzania To Mitigate Low Fertilizer Application Caused Food Insecurity**

### **Introduction:**

The invention of synthetic nitrogen fertilizer was one of, if not, the most profound inventions in the modern age. Directly responsible for the birth of 3.5 billion people, the Haber-Bosch process revolutionized agriculture, increasing crop yields by rates upwards of 60% annually (Mamnabi et al.).

The world is still reliant on synthetic fertilizers, as nitrogen fertilizer supports half the world's population's food production. But, in Tanzania, the crop productivity and yields are much lower in comparison to the developed world, as the average application rate in Tanzania is half the world's average. The core reason is that fertilizer in sub-Saharan Africa is four times as expensive in comparison to Europe ("The World's"), leading to 7% of the 33 million in the region having access to fertilizers.

These prices are only rising due to the crisis in Ukraine, but this issue can be mitigated through the use of microalgae-bacteria consortia and anaerobic digesters to convert the abundant organic waste located in Tanzania into a low-cost, environmentally sustainable, and nutrient dense fertilizer.

### **Background:**

Officially known as the United Republic of Tanzania, the country is located on the border of Uganda, Kenya, Mozambique, Malawi, Zambia, Rwanda, Burundi, and the Democratic Republic of the Congo. It is home to 63.6 million people, who are ruled under a one-party system established in 1977.

A tenth of Tanzania's total land mass is suitable for farming (MacroTrends) which has led to Tanzania's GDP being made up mostly by its agriculture, with the sector employing over 75% of all households in the developing nation (USAID), with the main exports being maize, cotton, sugar, sweet potatoes, and coffee (Kramer).

Over 49% of all families live below the wage of \$1.90 per day, leaving many families to starve out of poverty. 48% of all Tanzanians were undernourished in 2019, with 34% of all children under the age of five having their growth stunted, and 45% of women of reproductive age being anemic (USAID). The food crisis in Tanzania is one of the worst in the developing world, and its scale will only grow due to the economic downturn caused by the pandemic and the crisis in Ukraine.

**Issue:**

Despite being one of the hungriest nations, Tanzania has the potential to be one of the strongest regional exporters of crops. Named third strongest candidate for agricultural growth within sub-Saharan Africa in an assessment by McKinsey & Company in partnership with the World Bank (Goedde et al.), Tanzania contains a significant portion of the arable land in Africa, which itself houses 60% of the world's total arable land (Jayne et al.). And, unlike many other nations in the region, Tanzania's agriculture industry sees moderate support from the government and population where over 75% households are involved in farming, but the nation still cannot produce enough crop to curb its own hunger.

This is due to one simple reason: Tanzania's fertilizer application rate, specifically its extremely low synthetic nitrogen fertilizer application rate per hectare.

**Fertilizers:**

In order for crops to grow efficiently, crops need sixteen essential nutrients. Carbon, hydrogen, and oxygen are widely available to crops as they can be accessed through water and carbon dioxide in the atmosphere. The rest of the essential nutrients are absorbed through the soil crops are grown in. They are divided into the categories of micronutrients, secondary nutrients, and macronutrients. The majority of fertilizers focus on providing the nutrients in the macronutrient category: nitrogen, potassium, phosphorus, and potassium (Savci). Nitrogen is the most important nutrient for crop productivity.

Fertilizers can be broken down into two categories: organic and inorganic. Organic fertilizers are derived completely from plant or animal matter. Inorganic fertilizers, also known as synthetic fertilizers, are the most efficient and common fertilizers. Derived from nitric acid, sulfur, and atmospheric nitrogen, these fertilizers are responsible for the birth and sustainment of 3.5 billion people. They are manufactured through the Haber-Bosch process, which converts hydrogen and nitrogen into ammonia, emitting 6 tons of carbon for every ton of ammonia produced as a byproduct, almost 2% of the global carbon dioxide emissions (Ghavam et al.).

Nitrogen fertilizer application per hectare is the number one source of agricultural productivity worldwide. The larger the population and food demand, the higher amount of nitrogen fertilizer applied per hectare. The global average of nitrogen fertilizer application rates is 69.78 kg per hectare, with developed countries that host comparable population sizes to Tanzania having application rates within the range of 60 kg per hectare to 64 kg per hectare ("Nitrogen fertilizer").

Tanzania's nitrogen fertilizer application rate is 9.04 kg per hectare, less than 13% of the global average, and low by regional standards.

The main reason behind the lack of application is the extremely high prices for fertilizers in Africa as a whole, with fertilizers being four times more expensive in Africa than they are in other parts of the globe.

The constant political and economic instability in the region along with rampant corruption creates unaccounted tolls at official and unofficial border crossings for 80% of fertilizer shipments in the continent (Bensassi and Jarreau). This can hike up the costs by almost 20%, making a Central African Republic shipment to Tanzania more expensive than a North American shipment to Tanzania ("How to boost"). Along with this, Africa supplies 80% of its fertilizer through importation, which leaves it subject to volatile price increases in times of shortage (Minde et al.). The crisis in Ukraine and the halting of exports from Russia and the region as a whole has exacerbated this volatility, with 40% of Africa's fertilizer imports being halted, and sub-Saharan fertilizer prices doubling (Hassen and Bilali).

Currently, the region is undersupplied by over 350,000 metric tons, leading to average fertilizer use levels to plummet by 30%, meaning that crops for 100 million people will not be able to be grown (Good).

### **Economic ‘Solutions’:**

One of the most widely backed ideas for increasing affordable access to nitrogen fertilizers is the expansion of fertilizer manufacturing companies within Tanzania, and around Africa as a whole. Africa holds 9% of the natural gas reservoirs, so it could use this natural gas to support a synthetic fertilizer industry, which would decrease reliance on imports, thereby decreasing fertilizer prices, especially when in combination with fertilizer subsidies ("Natural gas in Africa").

This is only a short term solution, though. The majority of global superpowers have made pledges towards sustainability, and increasing synthetic fertilizer applications in Tanzania and around sub-Saharan Africa in general would significantly increase the amount of carbon dioxide and other greenhouse gas emissions. The Haber-Bosch process is widely known to produce carbon dioxide as a byproduct, but not widely discussed is the fact that half of the synthetic nitrogen fertilizer that is applied to crops gets broken down by microbes within the soil these crops are being grown in, releasing nitrous oxide, a greenhouse gas 300 times stronger than carbon dioxide (Tully et al.). Synthetic fertilizers also destabilize ecosystems, economies, and food systems even further. After initial applications to crops, a significant amount of synthetic fertilizers are carried to bodies of water through runoff, which can cause algal blooms through eutrophication, damaging the developing fishery industry in Tanzania and other coastal African countries.

But there is another aspect of synthetic nitrogen fertilizer that is not being discussed enough: its effect on soil degradation.

75% of all of sub-Saharan Africa’s soil is already degraded, and introducing synthetic fertilizers would only make this problem worse (Tully et al). If synthetic fertilizers were applied to this degraded soil, the soil’s pH would rise, and wouldn’t be able to absorb sufficient nutrients. Even carbon, which is not put in fertilizer because it is so readily available to crops, would be in limited access to crops as synthetic fertilizers decrease organic matter within soil (Lin et al.). Creating access to synthetic nitrogen fertilizers will not only put Tanzania into a worse position when the nation is pressured to change its fertilizer production method, but also create conditions for low crop productivity in the already hungry nation.

A solution that decentralizes fertilizer production, is low-cost, and environmentally sustainable would be to start utilizing the abundance of agricultural, municipal, and industrial waste in Tanzania and around Africa to create constant supply of organic fertilizers through the use of microalgae-bacteria consortia enriched anaerobic digestate.

### **Anaerobic Digestate and Microalgae-Bacteria Consortia:**

Anaerobic digestate is created through a process called anaerobic digestion. In this process, organic matter is broken down by acidogenic bacteria in the absence of oxygen. The process of anaerobic digestion takes around a month, and starts with the hydrolysis of the organic matter, where the acidogenic bacteria breaks down whatever type of organic molecule into whatever macromolecule the organic molecule is composed of. After, the bacteria utilizes the macromolecules to form intermediate volatile fatty acids, creating ammonia as a byproduct (Pramanik et al.). The bacteria then changes the volatile fatty acids into acetate, and the fatty acids into propionate, creating hydrogen as a byproduct. Finally the acetate and the propionate are converted into methane (Raspoor et al.).

The end of this process yields an energy source composed of 60% methane and 40% carbon dioxide called biogas, an energy source that can be used for almost anything. But, for the purposes of creating a fertilizer, the focus will be on the byproduct of the reaction, called digestate.

Digestate is composed of two parts, one liquid, one solid. Nitrogen is concentrated in the liquid part and phosphorus is concentrated in the solid part. Despite being nutrient rich, it is often thrown out by farmers, mainly due to the minimal amount of nitrogen and the form of the nutrients in the digestate. The macronutrients in synthetic nitrogen fertilizers are immediately available to plants, being available to crops less than a day after application due to the macronutrients being in a water soluble form (Suleiman et al.). The macronutrients in digestate are bonded to other elements, making them water insoluble, with crops taking up to a year to use them (Claassen and Carey).

The nutrient concentration in digestate is significantly less than synthetic fertilizer. Ammonia has a nitrogen concentration of 34%, diammonium phosphate has a phosphorus concentration of 18%, and potassium chloride has a potassium content of 14%. Digestate has nitrogen concentrations in the range of 1-3%, phosphorus concentrations in the range of 0.5-2%, and potassium concentrations in the range of 0.5-2% (Barlóg et al.).

There are also problems with the uniformity of digestate-based fertilizers. Digestate's consistency is dependent on the composition of the organic matter used, amount of organic matter used, the temperature outside of the anaerobic digester, and pressure in the anaerobic digester (Wairegi and Van Asten). The amount of nutrients in each batch of digestate will change depending on these factors, so farmers have much more difficult times adjusting to how much they should apply to their crops, in comparison to applying uniform synthetic nitrogen fertilizer.

The potential for digestate is immense, as it has decreased synthetic fertilizer use by 40% in some areas (Baştapak and Koçar). But digestate can be enhanced to be an effective fertilizer through the addition of microalgae to the already present bacteria. Microalgae have the ability to convert broken down nutrients into forms that are readily usable for plants while also using photosynthesis to produce biomass. When used to break down organic matter in combination with bacteria, the microalgae releases nutrients while producing biomass as the bacteria breaks down the biomass, releasing more nutrients (Jia et al.). When microalgae and bacteria are grown together in this matter, it is referred to as a consortium.

Research has proven the following increases in nutrient content depending on the microalgae and bacteria used: A *Chlorella vulgaris*-*Bacillus* sp. consortium lead to a phosphate increase by 10%, an ammonium increase by 3%, and a nitrate increase by 17% (*Growth of three*); a *Scenedesmus* sp.-*Enterobacter* sp. consortium lead to a nitrogen increase of 13% (Foladori et al.); and a *Chlorella vulgaris*-*Pseudomonas* sp. consortium lead to a phosphorus increase by 21.8%, a sulfur increase by 21%, and a nitrogen increase by 30% (Weimers et al.). When averaged, this increase in nutrient concentration would lead to nitrogen levels rising from 1-3% to 31-33%, phosphorus levels rising from 0.5-2% to 10.5-12%, and potassium levels increasing from 0.5-2% to 9.5-11.5%. This would be in the same range as many synthetic nitrogen fertilizers, which have nitrogen levels of 34-46%, phosphorus levels of 11-18%, and potassium levels of 8-14%, while emitting no carbon, being low-cost, decentralized, and only 20-25 days for the process to occur ("Nutrient recovery").

Small-scale/commercial anaerobic digesters are already available through companies like Anaergia and The Waste Transformer, with only an extra processing compartment being needed to allow the development of a microalgae-bacteria consortium. By processing and enriching the abundance of agricultural, municipal, and industrial organic waste within all of Africa through microalgae bacteria consortia, Tanzania would get access to environmentally sustainable, low-cost, and effective fertilizers to curb the nation's widespread food insecurity.

## Sources

- Barlóg, Przemysław, et al. "Yield, content and nutrient uptake by winter wheat and spring barley in response to applications of digestate, cattle slurry and NPK mineral fertilizers." *Archives of Agronomy and Soil Science*. Taylor & Francis Online, <https://doi.org/10.1080/03650340.2019.1676890>. Accessed 20 Feb. 2023.
- Baştabak, Benginur, and Günnur Koçar. "A review of the biogas digestate in agricultural framework." *Journal of Material Cycles and Waste Management*, vol. 22, 26 May 2020, pp. 1318-27. *SpringerLink*, <https://doi.org/10.1007/s10163-020-01056-9>. Accessed 20 Feb. 2023.
- Behaghel, Luc, et al. *The Impact of Farmer-to-Farmer Training on Agricultural Productivity in Uganda*. Edited by Karen Macours, J-PAL Africa, June 2018. *J-PAL*, [www.povertyactionlab.org/evaluation/impact-farmer-farmer-training-agricultural-productivity-uganda](http://www.povertyactionlab.org/evaluation/impact-farmer-farmer-training-agricultural-productivity-uganda). Accessed 19 Feb. 2023.
- Bensassi, Sami, and Joachim Jarreau. "Price discrimination in bribe payments: Evidence from informal cross-border trade in West Africa." *World Development*, vol. 122, Oct. 2019, pp. 462-80. *ScienceDirect*, <https://doi.org/10.1016/j.worlddev.2019.05.023>. Accessed 19 Feb. 2023.
- Claassen, V. L., and J. L. Carey. "Comparison of slow-release nitrogen yield from organic soil amendments and chemical fertilizers and implications for regeneration of disturbed sites." *Land Degradation & Development*, vol. 18, no. 2, 19 Dec. 2006. *Wiley Online Library*, <https://doi.org/10.1002/ldr.770>. Accessed 20 Feb. 2023.

Dzanku, Fred M., et al. "Yield Gap-Based Poverty Gaps in Rural sub-Saharan Africa." *World Development*, vol. 67, Mar. 2015, pp. 336-62. *ScienceDirect*,  
<https://doi.org/10.1016/j.worlddev.2014.10.030>.

Foladori, P., et al. "Evolution of real municipal wastewater treatment in photobioreactors and microalgae-bacteria consortia using real-time parameters." *Chemical Engineering Journal*, vol. 345, 1 Aug. 2018, pp. 507-16. *ScienceDirect*,  
<https://doi.org/10.1016/j.cej.2018.03.178>. Accessed 20 Feb. 2023.

Ghavam, Seyedehhoma, et al. "Sustainable Ammonia Production Processes." *Frontiers Energy Research*, vol. 9, 9 Mar. 2021. *Frontiers*, <https://doi.org/10.3389/fenrg.2021.580808>.

Goedde, Lutz, et al. "Winning in Africa's agricultural market." *McKinsey*, McKinsey & Company, 15 Feb. 2019,  
[www.mckinsey.com/industries/agriculture/our-insights/winning-in-africas-agricultural-market](http://www.mckinsey.com/industries/agriculture/our-insights/winning-in-africas-agricultural-market). Accessed 6 Feb. 2023.

Good, Keith. "High Fertilizer Prices Impacting Developing Countries and Europe- Food Price Repercussions a Concern." *Farm Policy News*, UIowa, 23 Jan. 2022,  
[farmpolicynews.illinois.edu/2022/01/high-fertilizer-prices-impacting-developing-countries-and-europe-food-price-repercussions-a-concern/](http://farmpolicynews.illinois.edu/2022/01/high-fertilizer-prices-impacting-developing-countries-and-europe-food-price-repercussions-a-concern/). Accessed 19 Feb. 2023.

*Growth of three microalgae strains and nutrient removal from an agro-zootechnical digestate*. Vol. 92, no. 6, July 2013. *ScienceDirect*,  
<https://doi.org/10.1016/j.chemosphere.2013.04.023>. Accessed 20 Feb. 2023.

Hassen, Tarek Ben, and Hamid El Bilali. "Impacts of the Russia-Ukraine War on Global Food Security: Towards More Sustainable and Resilient Food Systems?" *Impacts of the Russia-Ukraine War on Global Food Security: Towards More Sustainable and Resilient*

*Food Systems?* MDPI, <https://www.mdpi.com/2304-8158/11/15/2301#>. Accessed 19 Feb. 2023.

"How to boost fertilizer use by African smallholders." *IFAD*, [www.ifad.org/en/web/latest/-/news/how-to-boost-fertilizer-use-by-african-smallholders-](http://www.ifad.org/en/web/latest/-/news/how-to-boost-fertilizer-use-by-african-smallholders-). Accessed 19 Feb. 2023.

Jayne, T.S., et al. "Land pressures, the evolution of farming systems, and development strategies in Africa: A synthesis." *Food Policy*, vol. 48, Oct. 2014, pp. 1-17. *ScienceDirect*, <https://doi.org/10.1016/j.foodpol.2014.05.014>. Accessed 19 Feb. 2023.

Jia, Huijun, et al. "Removal of nitrogen from wastewater using microalgae and microalgae–bacteria consortia." *Cogent Environmental Issue*, vol. 2, no. 1, 2016. *Taylor & Francis Online*, <https://doi.org/10.1080/23311843.2016.1275089>. Accessed 20 Feb. 2023.

Kramer, Lars. "Number of households engaged in agricultural activities in Tanzania between October 2019 and September 2020, by area." *Statista*, 26 Jan. 2023, [www.statista.com/statistics/1258579/number-of-households-in-agriculture-in-tanzania-by-area/](http://www.statista.com/statistics/1258579/number-of-households-in-agriculture-in-tanzania-by-area/). Accessed 6 Feb. 2023.

Lin, Weiwei, et al. "The effects of chemical and organic fertilizer usage on rhizosphere soil in tea orchards." *PLoS One*, 28 May 2019. *PubMEDCentral*, <https://doi.org/10.1371/journal.pone.0217018>. Accessed 20 Feb. 2023.

*Macrotrends*. Macrotrends LLC, [www.macrotrends.net/countries/ZAF/tanzania/arable-land](http://www.macrotrends.net/countries/ZAF/tanzania/arable-land). Accessed 6 Feb. 2023.

Malpass, David. "A transformed fertilizer market is needed in response to the food crisis in Africa." *World Bank*, World Bank Group, 21 Dec. 2022,

[blogs.worldbank.org/voices/transformed-fertilizer-market-needed-response-food-crisis-africa](https://blogs.worldbank.org/voices/transformed-fertilizer-market-needed-response-food-crisis-africa).

Mamnabi, Sohrab, et al. "Improving yield-related physiological characteristics of spring rapeseed by integrated fertilizer management under water deficit conditions." *Saudi Journal of Biological Sciences*, vol. 27, no. 3, Mar. 2020. *ScienceDirect*, <https://doi.org/10.1016/j.sjbs.2020.01.008>.

Minde, Isaac J., et al. "Promoting Fertilizer Use in Africa: Current Issues and Empirical Evidence from Malawi, Zambia, and Kenya." *Michigan State University Food Security Collaborative Working Papers. AgEcon Search*, <http://dx.doi.org/10.22004/ag.econ.54934>. Accessed 19 Feb. 2023.

"Natural gas in Africa: Why fossil fuels cannot sustainably meet the continent's growing energy demand." *Climate Action Tracker*, 30 May 2022, [climateactiontracker.org/publications/natural-gas-in-africa-why-fossil-fuels-cannot-sustainably-meet-the-continents-growing-energy-demand/#:~:text=Africa%20is%20home%20to%20nearly,6%25%20of%20global%20natural%20gas](https://climateactiontracker.org/publications/natural-gas-in-africa-why-fossil-fuels-cannot-sustainably-meet-the-continents-growing-energy-demand/#:~:text=Africa%20is%20home%20to%20nearly,6%25%20of%20global%20natural%20gas). Accessed 19 Feb. 2023.

"Nitrogen fertilizer use per hectare of cropland, 1961 to 2019." *Our World In Data*, [ourworldindata.org/grapher/nitrogen-fertilizer-application-per-hectare-of-cropland?tab=chart&country=~TZA](https://ourworldindata.org/grapher/nitrogen-fertilizer-application-per-hectare-of-cropland?tab=chart&country=~TZA). Accessed 6 Feb. 2023.

"Nutrient recovery from anaerobic digestion of food waste: impacts of digestate on plant growth and rhizosphere bacterial community composition and potential function in ryegrass." *Biology and Fertility of Soils*, vol. 56, 26 May 2020, pp. 973-89. *SpringerLink*, <https://doi.org/10.1007/s00374-020-01477-6>. Accessed 20 Feb. 2023.



Oviedo, Jineth Arango, et al. "A half-century of research on microalgae-bacteria for wastewater treatment." *Algal Research*, vol. 67, Sept. 2022. *ScienceDirect*, <https://doi.org/10.1016/j.algal.2022.102828>. Accessed 20 Feb. 2023.

Pramanik, Sagor Kumar, et al. "The anaerobic digestion process of biogas production from food waste: Prospects and constraints." *ScienceDirect. ScienceDirect*, <https://doi.org/10.1016/j.biteb.2019.100310>. Accessed 20 Feb. 2023.

Raspoor, M., et al. "Recognizing the challenges of anaerobic digestion: Critical steps toward improving biogas generation." *Fuel*, vol. 261, 1 Feb. 2020. *ScienceDirect*, <https://doi.org/10.1016/j.fuel.2019.116497>. Accessed 20 Feb. 2023.

Savci, Serpil. "Investigation of Effect of Chemical Fertilizers on Environment." *APCBEE Procedia*, vol. 1, 2012, pp. 287-92. *ScienceDirect*, <https://doi.org/10.1016/j.apcbee.2012.03.047>. Accessed 19 Feb. 2023.

Seleiman, Mahmoud F., et al. "Chemical composition and in vitro digestibility of whole-crop maize fertilized with synthetic fertilizer or digestate and harvested at two maturity stages in Boreal growing conditions." *AFSci*, vol. 26, no. 1, 4 Apr. 2017. *Scientific Agricultural Society of Finland*, <https://doi.org/10.23986/afsci.60068>. Accessed 20 Feb. 2023.

"Tanzania." *GlobalEdge*, Michigan State University, [globaledge.msu.edu/countries/tanzania/economy](http://globaledge.msu.edu/countries/tanzania/economy). Accessed 6 Feb. 2023.

"Tanzania: Agriculture and Food Security." *USAID*, United States Agency for International Development, [www.usaid.gov/tanzania/agriculture-and-food-security](http://www.usaid.gov/tanzania/agriculture-and-food-security). Accessed 6 Feb. 2023.

"Tanzania Hunger Statistics 2001-2023." *Macrotrends*, [www.macrotrends.net/countries/TZA/tanzania/hunger-statistics](http://www.macrotrends.net/countries/TZA/tanzania/hunger-statistics). Accessed 6 Feb. 2023.

- Tully, Katherine, et al. "The State of Soil Degradation in sub-Saharan Africa: Baselines, Trajectories, and Solutions." *Sustainability*, vol. 7, nos. Enhancing Soil Health to Mitigate Soil Degradation, 26 May 2015. *MDPI*, <https://doi.org/10.3390/su7066523>. Accessed 20 Feb. 2023.
- Wairegi, L. W.I., and P. J.A. Van Asten. "The agronomic and economic benefits of fertilizer and mulch use in highland banana systems in Uganda." *Agricultural Systems*, vol. 103, no. 8, Oct. 2010. *ScienceDirect*, <https://doi.org/10.1016/j.agsy.2010.06.002>. Accessed 20 Feb. 2023.
- Ward, Alastair J., et al. "Optimisation of the anaerobic digestion of agricultural resources." *Bioresource Technology*, vol. 99, no. 17, Nov. 2008. *ScienceDirect*, <https://doi.org/10.1016/j.biortech.2008.02.044>. Accessed 4 Feb. 2023.
- Weimers, Kristina, et al. "Liquid Anaerobic Digestate as Sole Nutrient Source in Soilless Horticulture—Or Spiked With Mineral Nutrients for Improved Plant Growth." *Plant Science*, vol. 13, 23 Mar. 2022. *Frontiers*, <https://doi.org/10.3389/fpls.2022.770179>. Accessed 20 Feb. 2023.
- "The World's Most Expensive Fertilizer Market: sub-Saharan Africa." *Gro Intelligence*, 1 Dec. 2016, [gro-intelligence.com/insights/fertilizers-in-sub-Saharan-africa](https://gro-intelligence.com/insights/fertilizers-in-sub-Saharan-africa). Accessed 4 Feb. 2023.
- Xu C, Huang S, Tian B, Ren J, Meng Q, Wang P. Manipulating Planting Density and Nitrogen Fertilizer Application to Improve Yield and Reduce Environmental Impact in Chinese Maize Production. *Front Plant Sci*. 2017 Jul 12;8:1234. doi: 10.3389/fpls.2017.01234. PMID: 28747925; PMCID: PMC5506086.