

## Electortransformation of *Boletus Edulis*

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**Doctor of Medical Sciences** (Saratov State Medical University, the USSR Research Institute for Antibiotics, the All-Russian Center for Emergency and Radiation Medicine - the last Doctoral degree is not available in the US). **CURRENTLY:** CEO, Executive Chairman, President of Microbial Biocatalyst International, Inc. and Inorgcarbodiesel, Inc. Carbon Negative technology (reduce the air CO<sub>2</sub> levels to the pre-petroleum era) and the use of the air CO<sub>2</sub> to manufacture Carbon Negative fuels and Carbon Negative fuel mixtures suitable for use by cars / trucks having Gasoline or Diesel fuel motors) due to the loss of the fresh water vapors to the Space. Global food shortage is coming in the next 10-25 years. The Author of 226 peer-reviewed publications; 109 artificial DNA sequences deposited to NCBI; chapters in 23 published books, two books are published and on sale at [Amazon.com](https://www.amazon.com); 24 patents / patent applications.

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### Abstract

We have developed for the first time the electrotransformation procedure of valuable food source *Boletus edulis* for anticipated in the next 10-25 years global shortness of the fresh water sucked by the Space vacuum and therefore leaving Earth at the rate of ~25,920 liters per day, or 9,467 m<sup>3</sup> per year. Said fresh water loss of will cause Global starvation since no one will grow crops and livestock without fresh water. To create genetically engineered foods, we used our unique Electrofusion / Electrotransformation Generator. The electrotransformation procedure is reliable offering up to 10<sup>6</sup> transformants per microgram of the transforming recombinant DNA isolated from *E. coli* as the intermediate transforming DNA host.

**Keywords:** Electrotransformation, Anticipated Fresh Water Shortage, Anticipated Shortage of Crops and Livestock Manufacture, Genetically Engineered Foods Sold at Our New Type of Gas Stations, Expression of Recombinat Genes in White Tubular Mushroom of *Borovik Family*

### 1. Introduction

This is the first ever report of the electrotransformation of intact cell forms of white tubular mushroom *Boletus edulis* (also called porcini, king bolete, or cèpe) which is a wonderful mushroom (delicatessen) for cooking in each country of the World. Our task was to develop this electrotransformation (ET) procedure since we have designed and accomplished introduction of multiple recombinant genes into white tubular mushroom *Boletus edulis* which is always the ultimate target for any person collecting

mushrooms as their hobby. At our corporations we have succeeded to create recombinant mushrooms *Boletus edulis* expressing bovine myoglobin, sheep myostatin, tomato genes, potato genes, pepper genes, bread (wheat) genes and some genes of beans. Besides outstanding nutritional qualities of *Boletus edulis* it has wonderful therapeutic characteristics which we will plan to use in our corporate future [1].

Recent studies have proved the essential role of gut microbiota in

human health, including its involvement in metabolizing nutritional compounds, maintaining a healthy digestive tract and regulating the homeostasis of the immune system [1]. If this network is disrupted then pathogenic disorders and inflammations might develop. While antibiotics have been a worldwide game-changer in treating multiple infectious illnesses and prevent development of infected wounds at the human body the use of chemotherapeutic agents like antibiotics can have unintended consequences hindering the development of healthy bacteria and impacting multi drug resistant microbial strains within the digestive tract [2] and all other the human body [http://syngasbiofuelsenergy.com].

The reduction of gut microbiome diversity under the antibiotic treatment directly impacts the interactions between the host and microbes, the immune system's balance, and the host's protection against harmful microorganisms [3]. Furthermore, the human gut microbiota may alter the effectiveness of specific medicinal treatments [4]. The edible mushroom *Boletus edulis* (*porcini*) has outstanding nutritional and medicinal value. The nutritional value resides in the high content of carbohydrates, proteins, minerals, and flavor components while said mushrooms are low in calories and fat [5]. The medicinal qualities are linked to their content of bioactive substances, such as polysaccharides [6] which act by modulating normal gut microbiota. This indicates that, in the digestive tract, the polysaccharides function as prebiotics [7].

Prebiotics are the dietary elements that undergo selective fermentation, leading to changes in the composition and activity of the normal human microbiota in the gastrointestinal tract [8]. These changes provide the host with nutritional and health advantages. The majority of polysaccharides are found in *Boletus edulis* are  $\beta$ -glucan polymers, with the main chain consisting of  $\beta$ -(1 $\rightarrow$ 3) and  $\beta$ -(1 $\rightarrow$ 6) bounds. Furthermore, compounds such as chitin, mannans, galactans, and xylans are among the polysaccharides in *Boletus edulis* [9]. Because the digestive enzymes released by the stomach and pancreas cannot hydrolyze the  $\beta$ -glucosidic link, the non-digestible carbohydrates found in this mushroom can function as prebiotics in the digestive system. Recent research demonstrated that two prebiotic compounds, RoBioMush1 and RoBioMush2, can potentially modulate the microbiota associated with certain cardiovascular disorders [1,10,11].

Our task was to develop for the first time the electrotransformation procedure for the white tubular mushroom *Boletus edulis* intact cell forms (germinating spores) as we have done that much before when for the first time developed the electrotransformation procedure for antibiotic producing *Streptomyces* [13,14].

Intact spores do have the characteristics and fate of the intact bacterial cells suitable for the electrotransformation using our unique electrotransformation / electroporation Generator [15-17]. Indeed, we have elaborated the procedure to create the spore suspension for their germination in the nutrient medium making said spores suitable for the electrotransformation procedure using our unique electrotransformation / electroporation Generator [16,17].

## 2. Materials and Methods

In brief the procedure is the following. Under the horizontal laminar hood [18] we have got the mature spores of the white tubular mushroom *Boletus edulis* (250 g or slightly above that). We got the spores from the single *Boletus edulis* mushroom in their "tubula" (places where the mushroom spores are located) using the sterile disposable scalpel [19]. Said mature spore mass was transferred into the sterile beaker [19]. The beaker size depends on the mass of the mushroom. For the *Boletus edulis* of about 250 g mass the beaker size was 100 ml. For the bigger mushroom size, we recommend beakers of higher volume. The role of the beaker is to accommodate not only the separated from the mushroom spore mass in the tubules but also to accommodate the volume of the medium for the spore germination (12 times the volume of the isolated spore mass).

We used the following nutrient medium for the mushroom *Boletus edulis* spore germination process of the following composition, g/l:

Meat extract (Difco)	10.0
Soybean Extract (Sigma) [20]	10.0
Glucose	20.0
Na <sub>2</sub> HPO <sub>4</sub> 47.76 mM.(unhydrous dibasic)	6.78
KH <sub>2</sub> PO <sub>4</sub> 22,044 mM.(monobasic);	3.0
NaCl; 8.556 mM.	0.5
NH <sub>4</sub> Cl; 18.695 mM	1.0
Milli-Q water:	up to 1 L
pH	6.9 +_ 0.5

before autoclaving of said nutrient medium for 35 min at steam pressure in the autoclave of about 1.15 kg/cm<sup>2</sup>.

The medium was chilled to about 30 °C before its addition to the mass of spores / spores in tubules. The diluted with said medium spores / spores in tubules were incubated at 30°C for four-four and a half hours. After that we might anticipate that the spores started to germinate making the bacteria-like in their shape structures similar to those shown in Fig. 1. The structures shown in Fig. 1 do not exceed 1.0-1.5 micrometers in length (each).



**Figure 1:** The Germinating Spores of the White Tubular Mushroom *Boletus edulis*.

Before said nutrient medium addition the mass of tubules with spores is cut as much as possible with the sterile scalpel, mixed the content of the beaker using that scalpel several times to provide the homogeneity of the spores in tubules / spores mass with the added nutrient medium. For the mushroom weighting 250g we have added 80 ml of said medium. The added medium was mixed with the treated by sterile scalpel mass of the spores in tubules / spores and then was transferred to the 15 ml centrifuge tubes [20] by about 5- 6 ml. Said plastic centrifuge tubes were placed in the thermostated at 30°C shaker [21] set at 200 rpm shaking speed for 4 hours.

Upon finish of the shaking we collected the germinated spores of *Boletus edulis* by centrifugation of said tubes [22]. Collected germinating spores of *Boletus edulis* were aseptically transferred to the sterile 0.5 microliter polypropylene tubes served us as the ET cuvettes [16] for the use of our electrotransformation / electroporation Generator [14-16]. Said cuvettes were placed at the ice block [16,17] for the electrotransformation (ET) procedure discussed at [16,17] and subjected to the procedure of ET. The ET was conducted by 3.5 ms square pulses at the electric field strength of 12.5 to 14 kV/cm. Immediately after the ET the content of the ET cuvettes was diluted with 0.5 ml of the same medium used to germinate the spores of *Boletus edulis*.

The diluted suspension of the ET spores was then left at 30°C for 1 hour and then the content from each 0.5 ml ET cuvette was transferred into the 15 ml centrifuge tubes. The 5 ml of the same rich spore germination medium was added to each tube, pellets mixed with the medium and the tubes were transferred to the sterile incubated at 30°C cultivation boxes for inoculation of soil filled by 10 cm of the sterilized by autoclaving 6 times for 2 hours at 120°C (2 atm or kg/cm<sup>2</sup>) per each time of autoclaving. The inoculated

ET spores of the mushroom were incubated under said conditions in the autoclaved soil for 10 days soil with the high content of organics [23]. During that time we watched the development of the recombinant mushrooms comprising the recombinant genes of bovine myoglobin [24], sheep mystatin [24], tomato [25], potato [25], pepper [25], bread (wheat) [26] and Bush pinto beans [26].

### 3. Results

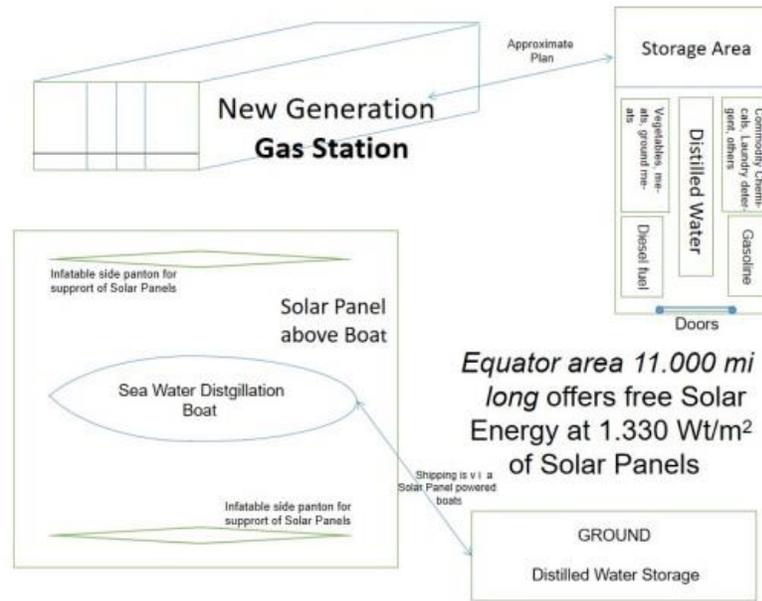
Under the inoculation conditions it is really hard to investigate the efficiency of our designed herein electrotransformation procedure. However, based on our earlier observation of the same procedure as that was related to both Gram Positive and Gram Negative microorganism the electrotransformation efficiencies were really high reaching of about 10<sup>11</sup>-10<sup>12</sup> transformants per each microgram of the transforming DNA in Gram Negative bacteria and of about 10<sup>6</sup>/microgram of transforming DNA for Gram Positive bacteria transformed with the same DNA isolated from Gram Negative bacteria. We never saw any of the cultivation boxes inoculated with the electrotransformed germinated spores of the white tubular mushroom *Boletus edulis* with less than 1,000 of growing mushrooms in each of the boxes installed into the special sterile incubation facility located at our corporate R&D Campus.. Said recombinant *Boletus edulis* mushrooms had to be re-inoculated into the other sterile boxes located at the same incubation facility to observe the full size mushrooms growing to take their pictures for our R&D records.

When the recombinant mushrooms were grown large enough (up to 250 g each) to taste them cooked or properly washed and sliced to make salads for the recombinants with the recombinant genes of the pepper, the tomato or the eggplants they were subjected to testing by the crowd of our corporate Senior Executives who were advised by the Author that the product they will test

is the genetically engineered food which has not been approved yet by the respective Governmental authorities. All our Senior Executives disregarded such our statement and have been eating said genetically engineered mushrooms well raw in the form of salads or cooked as shish kabobs [27] or boiled / steamed as the recombinant potato [28]. We must note herein that our genetically engineered mushrooms with kinds of taste of the real meats, vegetables and spices do not contain the alpha-Glucose and therefore are suitable for feeding people who already have developed either Diabetes I or Diabetes II.

#### 4. Discussion

As promised above we are ready to cope with the associated with the coming in the next 10-25 years shortness of the fresh water on our planet and related to that circumstance shortness of the manufacture of livestock and crops. We are ready to manufacture enough genetically engineered foods using our described herein strategy of using the Carbon Negative white tubular mushrooms where we will introduce the genes from meats, vegetables, spices, bread-like structures and beans. Other genetically engineered foods will also be produced at our corporate facilities but currently we do not have any idea on that kind of foods the customers would like to get at our new type of gas stations shown below in Fig. 2.

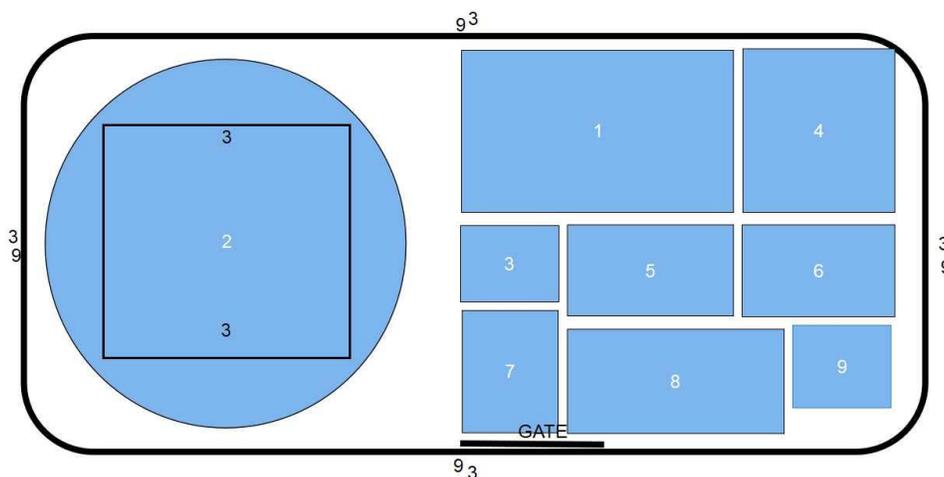


**Figure 2:** Our New Type of Gas Stations Selling Not Only the Carbon Negative Fuels but Also the Distilled Water We Will Produce in Abundance from The Sea Water and The Genetically Engineered Foods (After Their Approval by The Respective Governmental Agencies).

The distilled water will be produced using the salty water distillation using our high efficiency solar panels at the Equator areas of Earth. Due to the irregular solar light availability at different latitudes we prefer to use said areas for the sea water distillation process using our projected distillation boats with our high efficiency solar panels and the alkaline batteries we will purchase at the American shipyards from the decomposed submarines. Said boats will be powered by the alkaline batteries connected to the solar panels and the DC motors from the same shipyards as we plan to purchase at substantial discounts from the National shipyards decomposing the submarines of various types along with the DC motors from the same submarines to power our distillation and shipment boats /

ships. The question still exists how we would cope with the fresh water shortness by producing abundant amount of the distilled water for doing laundry and washing the bodies of our valued customers (it is under the discussion with our Senior Executives to develop the new proposals for our investors). We will publish that topic also as soon as we will get the economically sound solutions for this problem.

Said new type of the gas stations were in great agreement with our projected plan for the corporate blocks we will be using when get the proper investor funding (Figure:3).



**Figure 3:** The Proposed to the Author's investors Corporate Site Near Brownsville TX. The Numbers Are Consistent with The Numbers of Elements of the Corporate Business Center:

1. Corporate workplace for all corporate workers.
2. Corporate Housing Sector with the stadium area for those who prefers to run for health reasons.
3. Corporate Security Center with the side offices / areas for Corporate Security check up, Radar (see above) controlled perimeter of the Business Center.
4. *Carbon Negative fuels manufacturing area.*
5. *Carbon Negative fuels mixing / manufacturing area.*
6. Carbon Negative fuels mixing area.
7. *Corporate Area for Delivery of Food and Common Goods.*
8. *Corporate Garage with the cars / truck's maintenance facility.*
9. The Corporate Security personnel controlled **Corporate perimeter, Corporate entrance, Corporate food / goods delivery area; housing area; Corporate mixing area; Corporate manufacturing area; the site to manufacture DAA and Mesityl Oxide and mixing area with other Carbon Negative fuels; the area for cultivation of seeding of the genetically engineered mushrooms.**

Another topic comes out from our description of the genetically engineered foods production at our corporations. This is extremely important as it has to be done for any sort of Carbon Negative genetically engineered foods we will produce. The authorization of the Governmental authorities to use stated herein kinds of the new our genetically engineered foods we will produce at our corporations is mandatory.

#### Article Summary

- The expression system has been developed to express recombinant genes of various kinds of meat to manufacture Carbon Negative genetically engineered meats.
- Said system is based on the white tubular mushrooms known as Borovik.
- Said genetically engineered meats were as tasty as the real not genetically engineered meats as per the opinion of our top executive management team which has tasted the meals prepared at our corporation site.
- Said genetically engineered foods will be available at the new type of the gas stations to save plenty of time to our valuable customers.
- Said genetically engineered meats, wheta, vegetables, ect. have to be approved for mass consumption by the respective Governmental authorities.

#### Declaration of Interests Statement

##### Ethics approval and consent to participate

The Author has received all the proper documents granting the Ethical Approval and the Consent to Participate from the State of Texas officials. The Author has made sure that the ethical approval and his consent to participate in preparation and submission for publication of this article were properly approved by the respective authorities of the State of Texas.

##### Consent for Publication

The Author has expressed his complete consent to participate in work with this article and its publication in this Journal.

##### Availability of Data and Materials

The Author makes all his data and materials herein available for any third party. The data and materials might be obtained from the Author at PO Box 300230, Houston, TX, 77340. Email [professordrmichaelvtyurin@gmail.com](mailto:professordrmichaelvtyurin@gmail.com). If any third party needs any materials used to publish this article, please, do contact the Author.

##### Competing Interests

This article is another the Author's move in the war which has started by SHELL International Petroleum Corporation some time back by attempted murder of the Author. The people hired by

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SHELL has totaled the Author's corporate car and did some other illegal things to the Author which have concluded the Author that the war with the international petroleum corporations has started already. No Houston FBI or Houston Police investigation of said attempted murder has happened and this is behind the responsibility of the Texas Governor with all the legal remedies the Author might use in his fight to sue the State and its Governor Gregory Abbott. The attempted murder has the statute of limitations 20 years, and there are jurisdictions above the level of the State of TEXAS.

The Author had certain legal problems with the at that time Attorney General of the State of Texas in 2013, now Texas Governor, Greg Abbott and the Author is going to resolve all the legal problems with the Texas Governor, regardless would he be dismissed or not. The corporate Author's website <http://syngasbiofuelsenergy.com> was destroyed by the person, the Attorney of Hirsch and Westheimer Law Firm, PC by the last name Levy. She belongs to the same family which owned or owns the grocery stores chain named "Fiesta" in Houston Texas. The Author was unable to find a lawyer in Texas to file the respective federal law suit and recover his corporate website, but the Author has the US Constitutional right to recite this website in the references herein.

Therefore the Author has multiple legal problems with the State of Texas and the Author is going to resolve said legal problems at the respective Court level in the US. The Author is already involved into the war which has been started by the International Petroleum Corporation SHELL. The Author has approached SHELL offering them for the commercialization the proprietary technology of Gasoline or Diesel fuel manufacture from the air CO<sub>2</sub>, not from petroleum which SHELL and other international petroleum corporations use. SHELL representative met the Author, got his draft of the at that time in publication article on creation Gasoline from the air CO<sub>2</sub> [1,21-24,41,42]. Then SHELL hired two Mexicans to kill Dr. The Author in the car accident, paying the possibly around \$6,000 for this dirty job, which has been done in Houston TEXAS at the US59 down South (9494 Southwest Fwy).

The Houston FBI and the Houston Police were contacted by the Author multiple times over his mobile phone however there was no any detailed and thorough Police or the FBI investigation of said attempted murder of the Author since as the Author trusts, both Houston FBI and Houston Police are totally corrupted by the international petroleum corporations. Therefore any law is not used in the State of Texas to hurt said corporations. Therefore the Author has to look for the law enforcement outside of the State of Texas, at the National level and he will do that to affect the Texas Governor Abbott for his actions of 2013 and have him resign from his position. Therefore the Author has conflict of interests with the State of Texas, TEXAS Governor Gregg Abbott, Texas FBI and Texas Police. Since that time more scientific publications of the Author on the Gasoline and Diesel fuel production from the air CO<sub>2</sub> came out [15-49].

The US patents are extremely expensive, therefore the Author uses instead of patents scientific publications. Publications are as

good as the US patents, each the US patent has to claim something better than the Author already did in his scientific publications. The Author working on the Acetogens-biocatalysts has no any competition in the world because of his prior invention, the electroporation / electrofusion Generator, already sold as a sample (with no right for reproduction) to the US corporation BTX, Inc. /

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The Author has received no funding. The investors of the Author has read this article draft to his investors and they have decided not to include no funding information as they have stated to the Author. They stated the Author should write "No Funding" per this original article.

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### **Authors' Contributions**

The Author has conducted all the experiments himself. The Author has planned, wrote this original article and edited the written text, including proper placement of the illustrations mentioned which the Author owns. The Author read, edited and approved the final manuscript. The Author is the only owner of all materials disclosed in this original article. The Author has plans to distribute his proprietary products after their approval as needed. The Author might be contacted for the data and materials at PO Box 300230, Houston, TX, 77230, [professordrmichaelvtyurin@gmail.com](mailto:professordrmichaelvtyurin@gmail.com). The Author contributed to the study conception and design. Material preparation, data collection and analysis were performed by the Author. The first draft of the manuscript was written by the Author. The Author read and approved the final manuscript.

The Author has designed the ideology of this article by himself. The Author intends to develop the detailed structure and location of his Carbon Negative corporations to be in charge for the establishing and running testing trials of his inventions he plans to commercialize additionally to his major business of manufacture of Carbon Negative fuels and Carbon Negative genetically engineered foods for the Nationwide distribution.

The Author has conducted all the experiments himself. The Author has planned, wrote this original article and edited the written text, including proper placement of the illustrations mentioned which the Author owns. The Author read, edited and approved the final manuscript. The Author is the only owner of all materials disclosed in this original article. The Author has plans to distribute his proprietary products after their approval as needed. The Author might be contacted for the data and materials at PO Box 300230, Houston, TX, 77230, [professordrmichelvtyurin@gmail.com](mailto:professordrmichelvtyurin@gmail.com). The Author contributed to the study conception and design. Material preparation, data collection and analysis were performed by the Author. The first draft of the manuscript was written by the Author. The Author read and approved the final manuscript.

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The Author has designed the ideology of this article by himself. The Author intends to develop the special structure at his Carbon Negative corporations to be in charge for the establishing and running clinical trials of his inventions he plans to commercialize additionally to his major business of manufacture of Carbon Negative fuels and Carbon Negative genetically engineered foods for the Nationwide distribution.

The Author conceived of the presented idea. The Author developed the theory and performed the computations. The Author verified the analytical methods. The Author investigated therapeutic effects of “Mixture” and supervised the findings of this work. The Author discussed the results and contributed to the final manuscript. The Author carried out the experiment. The Author wrote the manuscript. The Author supervised the project. The Author conceived the original idea. The Author supervised the project. The Author developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. The Author contributed to the final version of the manuscript. The Author supervised the project.

The Author conceived and planned the experiments. The Author carried out the experiments. The Author planned and carried out the simulations. The Author contributed to sample preparation. The Author contributed to the interpretation of the results. The Author took the lead in writing the manuscript. The Author provided critical feedback and helped shape the research, analysis and manuscript. The Author designed the model and the computational framework and analyzed the data. The Author carried out the implementation. The Author performed the calculations. The Author wrote the manuscript. The Author conceived the study and were in charge of overall direction and planning. The Author designed and performed the experiments, derived the models and analyzed the data. The Author wrote the manuscript in consultation with other corporate employees, devised the project, the main conceptual ideas and proof outline.

The Author worked out almost all of the technical details, and performed the numerical calculations for the suggested experiment. The Author worked out the bound for quantum mechanics. The Author analyzed the data. The Author wrote the paper. The Author designed and directed the project; the Author performed the experiments; the Author analyzed spectra; the Author. made the simulations; the Author developed the theoretical framework; the Author wrote the article. The Author performed the measurements. The Author. was involved in planning and supervised the work, the Author processed the experimental data, performed the analysis, drafted the manuscript and designed the figures.

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### **Originality-Significance Statement**

The Author has wrote this original article based on his originality of the business approach and the existing resistance of the International Petroleum corporation to the technology of manufacturing carbon negative fuels to replace their production of fuels originating from petroleum. The reduction of the air CO<sub>2</sub> levels towards the pre-petroleum era of the year 1900 is paramount, since our planet loses fresh water to the outer Space vacuum. NASA has confirmed that in 2010 stating that the Earth has reached the “Point of No Return” to the healthy environmental conditions suitable for life on our planet. The new family of the gas stations selling not only Carbon Negative fuels but also foods for cooking at home and commodity chemicals for the households will save a lot of time for the customers of said new gas stations.

**The Author** Dr. Michael V. Tyurin is the Author of this original article. In 2017 after tragic death of his wife Prof. Dr. Tyurina Aleksandra Nicolaevna his father Prof. Dr. Tyurin Vladimir Inljich decided to change his first name from “Vladimir” to “Vladislav”. Therefore the Author Dr. MV Tyurin had to change his second name from “Vladimirovich” to “Vladislavovich”. ORCID # is 0000-0001-6943-6556. Contact address: Dr. Michael Vladislavovich Tyurin, CEO, Executive Chairman, President Microbial Biocatalyst International, Inc. and Inorgcarbiesel, Inc. PO Box 300230, Houston, TX 77230. Contact Email [professordrmichaelvturin@gmail.com](mailto:professordrmichaelvturin@gmail.com) .

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