UNIVERSIDAD DE LAS AMÉRICAS PUEBLA

Escuela de Ingeniería
Departamento de Ingeniería Química, Alimentos y Ambiental

Conceptual design of a pilot-scale biorefinery with *Lemna minor* from UDLAP's constructed wetland as feedstock

Tesis que, para completar los requisitos del Programa de Honores presenta la estudiante

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San Andrés Cholula, Puebla. Primavera 2021

Hoja de firmas

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Dedicatoria

A mis padres, motor y pilar en este camino, quienes creyeron en mí aún en esos días que yo no lo hacía.

Al Dr. René Reyes Mazzoco, ejemplo de persona e ingeniero para mí y alumnos de múltiples generaciones, por su gran apoyo.

Agradecimientos

Al Dr. René Lara Díaz, por su orientación, paciencia, y guía en este proyecto y a lo largo de toda la carrera.

A mis amigos y amigas, por hacer que este camino no fuera recorrido solo y llenarlo de risas y alegría.

A mis profesores y profesoras, por compartir su conocimiento, consejos y tener siempre la puerta de sus oficinas abierta para cualquier duda o inquietud.

A todos los integrantes de la Fundación Protectora de Puebla "Aurora Marín de Taboada", por ofrecer su apoyo desde el primer día que toqué sus puertas y por su increíble esfuerzo y dedicación por la educación en México.

Al departamento de Becas de la Universidad de las Américas Puebla.

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Nomenclature

A	*Lemna minor* wetland area, m^2
Be	Bioethanol potential, l/m^2
BeP	Bioethanol production, l/year
BdP	Biodiesel production, l/year
C	Capital cost per production unit, MXN/L or MXN/kW
d	Tractor working distance obtained from the project, km/year
E	Greenhouse gases emissions, g $CO_{2\ eq}$/MJ
G	Duckweed growth rate in dry mass, $g/(m^2*d)$
L	Lipid production, kg/year
LC	Lipid content, %
M	*Lemna minor* production, l/year
N	Number of data points, -
O	Operational cost per production unit, MXN/kW
S	Savings, MXN/year
t	Tractor working time obtained from the project, h/year
u	Cost per unit of biofuel, MXN/l
w	Energy density, MJ/l
$\dot{W}$	Power generation, kW

Greek symbols

ρ	Density, kg/l

Subindexes

Av	Average
Bd	Biodiesel
Be	Bioethanol
D	Diesel
DM	Dry matter
E	Ethanol
L	Lipid
Max	Maximum
Min	Minimum
M	Mitigation
$S1$	Section 1
$S2$	Section 2
T	Total

Acronyms

BOD	Biological Oxygen Demand
CAPEX	Capital costs
CONABIO	*Comisión Nacional para el Conocimiento y Uso de la Biodiversidad*
EPA	Environmental Protection Agency
GHG	Greenhouse gases
GWP	Global warming potential
IEA	International Energy Agency
MTBE	Methyl tert-butyl ether
NEB	Net energy balance
NER	Net energy ratio
OPEX	Operational and maintenance costs
SENER	*Secretaría de Energía*
UDLAP	*Universidad de las Américas Puebla*
WWTP	Wastewater treatment plant

Abstract

Fossil fuels represent the main source of energy both on a local scale and worldwide. Bioenergy has emerged as an alternative or complement to conventional energy. This document presents the conceptual design of a biorefinery using *Lemna minor* or duckweed from UDLAP's constructed wetland as feedstock. The hypothesis of the project is that Biofuel production from UDLAP's *Lemna minor* will bring positive environmental impacts. Duckweed has been proposed as biodiesel and bioethanol feedstock. A review of the four generations of biodiesel and bioethanol is given, talking about the advantages and controversies surrounding biofuels. Also, the important legislation in Mexico regarding energy transition and bioenergy is listed. A bibliographic research was carried out to evaluate the duckweed growth rate, lipid content, and bioethanol potential. A block diagram is proposed indicating the processes needed for the biofuel production from duckweed. It is estimated that from the area of UDLAP's wetland (400 m^2), an average production of 2,044.85 kg/year of dry biomass, 410.89 l/year of bioethanol and 194.66 l/year of biodiesel are expected. The estimated biorefinery's CAPEX and OPEX are 27,634.08 MXN and 1,577.14 MXN/year, respectively. The savings from the project would be 7,180.27 MXN/year having a return of investments around year five of operations. Also, with the project a GWP mitigation of ~0.9 ton CO_{2eq}/year is expected. Using the biofuels for a tractor it is expected to obtain 88.23 hours or 3,630.14 km of tractor work per year of operation. It is concluded that *Lemna minor* could be a viable and profitable feedstock for a biorefinery and has the potential for an upscaling or implementation of larger projects in Mexico.

Keywords: *biomass, bioenergy, Leman minor, duckweed, bioethanol, biodiesel, growth rate, biorefinery, conceptual design, constructed wetlands*.

1. Problem statement

In this section, a brief description of the project context and relevance is presented to justify a biorefinery implementation in Mexico. Also, the objectives, scope, and hypothesis are shown.

1.1. Introduction and justification

This subsection includes a general description of the energetic context, both on global and local scales. Some of the inconveniences of conventional fuels are presented.

1.1.1. Global energy situation

It is important to understand how much energy is being consumed and which are the energy sources being used. Also, it is important to analyze how these consumption trends have changed over time. Both global and local context are needed to find solutions to the present problematics.

The International Energy Agency or IEA (2020) on the Energy Balances Overview indicated that 14,282 Million Tonnes of Oil Equivalent (Mtoe) were produced in 2018 and included the distribution of the energy supply by its source (Figure 1). 82% of the energy came from fossil fuels; 27% from coal, 23% from natural gas and 32% from oil. On the other hand, biofuels, which could include bioethanol, biodiesel, biogas, among other fuels represented 9% of the total energy supply. This indicates that by far most of our energy comes from fossil fuels. While the specific distribution could change from one region to another, the overall trend remains the same worldwide.

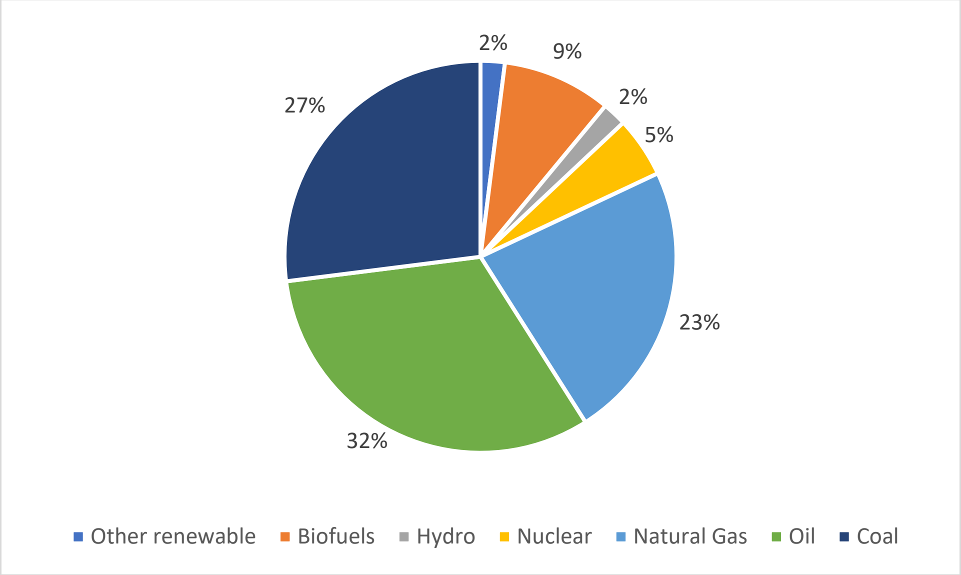


Figure 1. Total energy supply by fuel in 2018 (Based on IEA, 2020).

Another important aspect is how the energy consumption changes over time. On the same IEA (2020) document, it is stated that from 1971 to 2018 the energy supply grew 2.6 times, from 5,519 Mtoe to the previously mentioned 14,282 Mtoe. It is important to note that this change has not been constant, and it is getting faster. "*World energy consumption rose slightly more than 1 percent annually between 1970 and 2000, but between 2003 and 2007 the rate of growth jumped to nearly 5 percent per year.*" (Cunningham & Cunningham, 2012). So, while in general the energy production has been growing, in the last years, its growth has accelerated.

While the energy consumption has been changing, the overall energy supply distribution by fuel has not seen significant changes. In 1971 fossil fuels represented 86% of the total energy supply and biofuels 11% (IEA, 2020). 49 years ago, fossil fuels were the main energy source, and they still are.

It is also important to notice, that even though the fossil fuels reserves could be considered vast they still represent a finite resource. It is estimated that there are 10 trillion metric coal reserved, this is the equivalent of a supply for 200 years at current consumption rates, still some of this coal could be not economically recoverable. For oil, it is thought that there were 4 trillion barrels (bbl), but only half of them were considered recoverable. "*Of the 2 trillion recoverable barrels, roughly 1.26 trillion bbl are in proven reserves. We have already used more than 0.5 trillion bbl—almost half of proven reserves—and the remainder is expected to last 41 years at current consumption*." (Cunningham & Cunningham, 2012). For natural gas, the proven recoverable reserves are 6,200 trillion ft^3, that are equivalent to a supply of 60 years.

1.1.2. Mexico's energy situation

On a more local approach, some of the global tendencies are also reflected in Mexico. The Energy Secretary or SENER (2019) for its Spanish initials (*Secretaria de Energía*) presented the National Energy Balance (*Balance Nacional de Energía*) indicating key aspects of the energetic situation within the country. For the local energy production fossil fuels have a similar percentage as the global trend with 86.4% (SENER, 2019). But with a different distribution among coal, oil and natural gas; each having 4.3%, 62.4% and 19.7% (SENER, 2019); Mexico uses more oil and less coal than the global average. The biofuels would include the energy obtained from biomass and biogas, with a 5.7% and 0.1% of the national production (SENER, 2019), respectively; 5.8% in total. Figure 2 shows the complete distribution of primary energy production in Mexico.

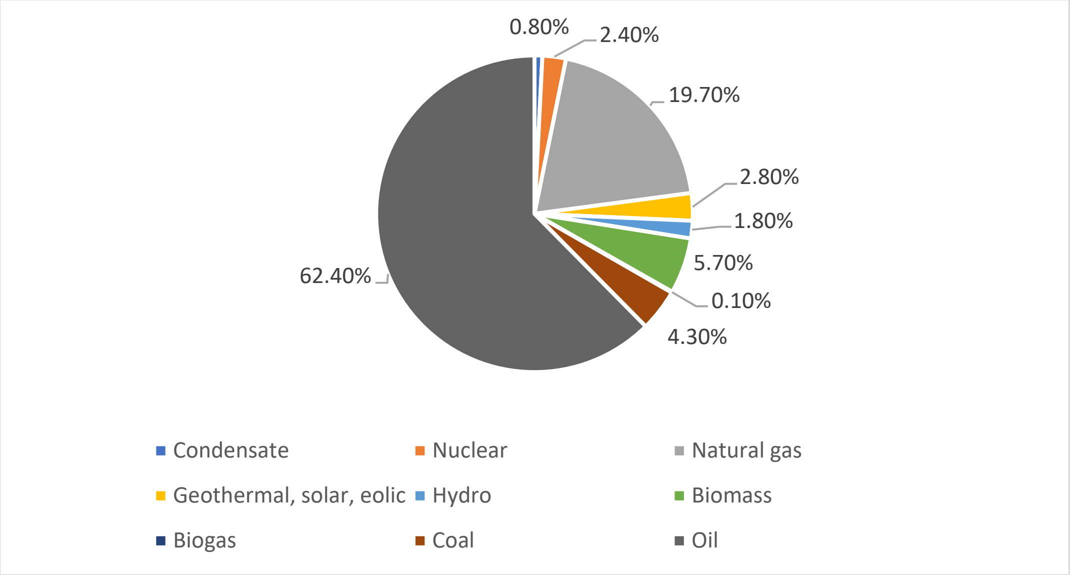


Figure 2. Primary energy production in Mexico, 2018 (Based on SENER, 2019).

Another parameter that the SENER (2019) considers important to evaluate the energy situation in the country is the energy independency, that represents how much of the energy demand is covered by national production. On one hand, the energy demand has increased from approximately 8,250 PJ to 9,236.86 PJ from 2008 to 2018 (SENER, 2019). On the other, the energy production dropped by 29.8% on the same period, reaching 7,027.22 PJ on 2018 (SENER, 2019). As a result of these two trends, since 2014 Mexico has consumed more energy than what it has produced within its territory (SENER, 2019). Regarding this deficit, most of the imported energy comes in form of fossil fuels such as gasoline, diesel, coke, fuel oil, etc. (SENER, 2019). Mexico needs energy projects with a wider diversification of their energy sources. And thus, aiming for less dependency on foreign markets and fossil fuels.

1.1.3. Conventional fuels inconveniences

While fossil fuels and conventional energy sources are in part responsible for the development and the worldwide industrialization, they have consequences that are worth mentioning. The use of conventional fuels or fossil fuels has environmental effects that have been reflected throughout the years. These effects come from the extraction of these resources to their consumption. The pollution could be to the soil, air, and water; thus, having a large range of impact.

One of the most significant environmental issues is the one caused by the Green House Gases, such as Carbon Dioxide (CO_2). The combustion of fossil fuels is one of the key contributors to this situation. "*International Energy Outlook 2016 predicted that the world energy-related carbon dioxide (CO_2) emissions will be increased by 10% in 2020 and 34% by the end of 2040*" (Marwa *et al*., 2017). Thus, it is important to find and use energy sources that, since their extraction to their usage, represent less damage to the environment.

Another concern regarding the fossil fuels is the effect that the spills or accidents that could be present on oil extraction or processing. An example of the effect of such event is found on the Deepwater Horizon accident. This explosion in 2010 of an oil platform on the Mexican Gulf led the death of 11 people and one of the largest oil spills in history (BP, 2010). "*The millions of gallons of oil that have spilled into the Gulf of Mexico are more like an epidemic, one that we will be fighting for months and even years…*" (Graham *et al*., 2011). This event jeopardized the life of different animals, such as sea turtles, fish, or other marine birds.

Still, there are attempts to mitigate or reduce the pollution emissions from fossil fuels. Such was the case of what happed in Mexico City around the 80's. In 1986 high concentration of lead was reported in the air (Cortez-Lugo *et al*., 2003). The use of the antiknock in commercial gasoline, Tetraethyllead, was the main reason of the high lead concentrations in the air. As a response, several initiatives were taken, among them the prohibition of the use of Tetraethyllead in gasoline produced by Pemex (Garza & Aragon, 1995; Pantic *et al*., 2018), also lead air emissions started to be regulated through a maximum limit of 1.5 $\mu g/m^3$ (DOF, 1993). As a result, the lead concentration in Mexico City is nearly zero nowadays (Cortez-Lugo *et al*., 2003).

As this case presented in Mexico City, there are actions worldwide that seek for a more responsible use of fossil fuels. On the next years, they would still be the main energy source around the world, but better processes, practices, legislations, and regulations could reduce the negative effect they have.

Still, the Methyl-tertbutyl-ether or commonly known as MTBE, the antiknock and oxidizer used in commercial gasoline in Mexico has controversies. MTBE could be a source of water pollution. In the USA, this compound was found in some of their ground waters, and even in small concentrations (20-40 ppb) it could make the water undrinkable due to its odor and taste (EPA, 2016). "*MTBE not only has high mobility in aquatic environment and drinking water system due to its solubility and lack of polarity, but also has resistance to decomposition for it is not significantly affected by microorganisms*" (Song *et al*., 2006). Is worth noticing, that while MTBE can be used in Mexico as 11% of gasoline blends (DOF, 2016), due to these concerns, some states in the USA have banned MTBA, e.g. New York and California (Song e*t al*., 2006; Cruz Serrano, 2018).

The MTBE used in the Mexican gasolines comes from the USA (Cruz Serrano, 2018). In 2018 PEMEX bought 25,000 barrels per day, almost 4 million liters per day. The consumption shows a rising tendency, from 2015 to 2018 Mexico acquisitions of MTBE rose by 31.5 % (Cruz Serrano, 2018).

1.2. Objectives

1.2.1. General objective

The overall research aim is to develop the conceptual engineering of a biorefinery using *Lemna minor* as feedstock.

1.2.2. Specific objectives

To address the main target, the following specific objectives have been carried out:

- Review Mexico's situation regarding energy consumption and production and the legislation involved on the biorefinery.
- Create the block diagram of the biorefinery.
- Evaluate the environmental and economic benefits of the project.

1.3. Scope

The scope of this thesis focusses only on the conceptual design of a biorefinery that uses the *Lemna minor* used on UDLAP's artificial wetland as biomass to produce biodiesel and bioethanol. The project takes into consideration the technical viability reported on different sources for the biofuel production and oil extraction from *Lemna minor*. The location of biorefinery is not proposed, since being the first project if this type the technical and economic feasibility are needed to be considered first.

1.4. Hypothesis

Biofuel production from UDLAP's *Lemna minor* will bring positive environmental impacts.

2. Theoretical framework

2.1. Mexico's legislation

In 2008 to diversify the energy sources in Mexico, the Law of Promotion and Development of Bioenergy Products (*Ley de Promoción y Desarrollo de Bioenergéticos*) was enacted. It looks forward to the promotion of the raw materials or inputs for bioenergy products from agricultural activities, algae, forestry, biotechnological and enzymatic processes, without jeopardizing the food safety. It also recognizes these activities to contribute to the rural development, improve life quality and reduce the pollutant emissions (DOF, 2008). In addition, it seeks for the promotion of research, production, and distribution of bioenergy products (DOF, 2008).

In 2015 the Law of Energy Transition (*Ley de Transición Energética*) was enacted. With it, the government looks forward to a gradual transition to cleaner energy sources. To do so, it promotes the use of renewable resources and waste to energy systems (DOF, 2015).

Other relevant regulation that is dictated by the Official Mexican Norm NOM-016-CRE-2016 (DOF, 2016). This NOM establishes that gasoline blends could have up to 5.8% of ethanol or up to 11% of MTBE as oxygenating and antiknock in Mexico; excluding the metropolitan areas of Mexico state, Monterrey and Guadalajara where no ethanol is allowed (DOF, 2016). This is important because this could limit the market.

Since this is a flammable product, NOM-076-SSA1-1993 proves the requirements for the use, process, storage, labeling, packaging, and transport of ethanol and diesel.

Finally, the guidelines for quality specifications and characteristics of bioethanol, biodiesel, and pure bioturbocine recommends bioethanol to have a minimum purity of 97.5%

ethanol (DOF, 2018). The same document indicates, among other traits, that biodiesel should have at least a cetane number of 47.

2.2. Biomass and biofuels

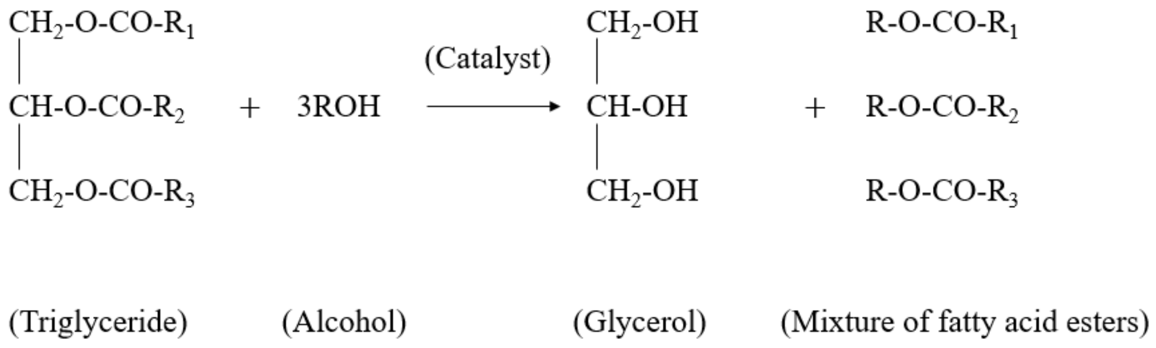
Most of the energy produced comes from fossil fuels (sections 1.1.1. & 1.1.2.), whoever some alternatives have emerged over the years as a substitution or complement of fossil fuels, including the bioenergy. "*Bioenergy is energy from biofuels. Biofuel is fuel produced directly or indirectly from biomass. Biomass is material of biological origin, for example wood, dung or charcoal and it excludes material embedded in geological formations and transformed to fossils.*" (FAO, 2021). Bioenergy could take advantage of the biomass that is currently unused such as urban organic waste or agro-industrial waste.

2.3. Biodiesel

Biodiesel is a direct substitute for conventional diesel, and in most cases can be used on engines designed for this fossil fuel. The most common practice is to mix biodiesel with diesel, the ratios could be 10:90 or 20:80. Biodiesel "*is defined as mono-alkyl esters derived from long chain fatty acids contained in animal fats and vegetable oils, and processed using alcohol and a catalyst.*" (Indra Riayatsuah *et al.*, 2017). The reaction used in biodiesel production is called transesterification (Figure 3). There are different factors that affect the transesterification reaction (Leung *et al.*, 2010) among them:

- Type of alcohol
- Type of catalyst
- Feedstock
- Temperature

- Reaction time
- Alcohol quantity



$$\begin{array}{lccccccl} CH_2\text{-O-CO-}R_1 & & & & CH_2\text{-OH} & & R\text{-O-CO-}R_1 \\ | & & & \text{(Catalyst)} & | & & \\ CH\text{-O-CO-}R_2 & + & 3ROH & \xrightarrow{\hspace{2em}} & CH\text{-OH} & + & R\text{-O-CO-}R_2 \\ | & & & & | & & \\ CH_2\text{-O-CO-}R_3 & & & & CH_2\text{-OH} & & R\text{-O-CO-}R_3 \end{array}$$

Figure 3. Transesterification reaction (Modified from Leung et al., 2010).

Some of the advantages of this fuel were mentioned by Ali and colleagues (2011). "*Biodiesel has several distinct advantages, over petroldiesel in that it is derived from a renewable source, reducing dependence on and preserving petroleum. It has high flash point leading to safer handling and storage, excellent lubricity, reduced exhaust emissions of particulate matter, VOC, CO, Green House gases and is biodegradable.*"

Therefore, it has brought attention for research and production. The interest in this biofuel has been reflected in the research and development of four generations of biodiesel each different depending on the feedstock used.

2.3.1. First generation biodiesel

The first generation of biodiesel refers to the biodiesel obtained from unused oils, like "*…those from soybeans, palm oil, sunflower, safflower, rapeseed, coconut and peanut…*" (Bhuiya *et al*., 2014). The oil is extracted from these sources, the crops are only designated to obtain oil for the biofuel production and the oil comes with a higher quality. In most cases the biofuel process starts from the crop's treatment to the oil extraction. In other cases, the oil is bought from a third-party supplier.

2.3.2. Second generation biodiesel

The second generation biodiesel is the one obtained from "*…non-edible vegetable oils, waste or recycled oil as well as animal fats…"* (Bhuiya *et al*., 2014). While some of the oil sources could overlap with the ones from the first generation, if the oil has been used it is considered as second generation biodiesel. This increases the reach of what can be considered a biofuel source. By using them, a smaller inversion is needed, since there is no need to plant or treat any crop, and a proper usage is given to what might have be considered as waste.

2.3.3. Third generation biodiesel

In recent years a third generation of biodiesel has been proposed, its oil sources are the algae. While the transesterification process could be very similar in all three generations, there are added benefits and difference on the raw materials and the process to obtain and treat them. So, it is important to analyze how could this new source help to the problematics the world is facing.

The algae are "*members of a group of predominantly aquatic photosynthetic organisms of the kingdom Protista. Algae have many types of life cycles, and they range in size from microscopic Micromonas species to giant kelps that reach 60 meters (200 feet) in length*" (Encyclopedia Britannica, 2018). For this process, the most commonly used type are the microalgae. Each type and specie can have different traits and could be grown under different conditions. Some of the species that have been used are: *Chlorella*, *Graesiella*, *Scenedesmus*, *Neochloris*, and *Nanochloropsis* (e.g. Dahiya, 2015, Carneiro *et al*., 2017, Marwa *et al*., 2017).

Also, microalgae can be divided into groups depending on their nutrition system. The microalgae that use sunlight and carbon dioxide are called Photoautotrophic. Like most plants, this algae group depends on the photosynthesis, and thus is limited by the sunlight. A second group, the heterotrophic cannot use the photosynthesis nor take advantage from the carbon inside Carbon dioxide molecules, as a result they need an organic Carbon source like sugar. Due to this they are limited by the carbon source, and this could increase the production cost. There is a third group called Mixotrophic that can us both type of nutritional systems.

There are three main cultivation systems, closed fermenters, photobioreactors and open ponds. Each has its advantages and disadvantages and could be used under certain conditions. The chosen one would also depend on the type of microalgae used. This leads to a variety of methods for the cultivation.

Photobioreactors are closed systems designed to provide the living organisms inside them with the proper light. Both sunlight and artificial light can be used. Photobioreactors are mainly used for photoautotrophic microalgae, due to their light demand. Photobioreactors provide a closed system with only controlled intervention to set the parameters as pH, temperature, present organisms, among other. They can be found with different designs, like tubular, flat plates, or tanks. Their downfalls could be related to the maintenance needed and their scalability. But photobioreactors provide a system with much more control on the process variables than open ponds.

Open ponds are open systems that are easier to maintain and construct and could be built on large land surface. Open ponds are also mainly used for photoautotrophic microalgae. Since they are open systems, there are environmental factors that could change the set parameters and affect the cultivation. But open ponds represent a smaller inversion than other projects.

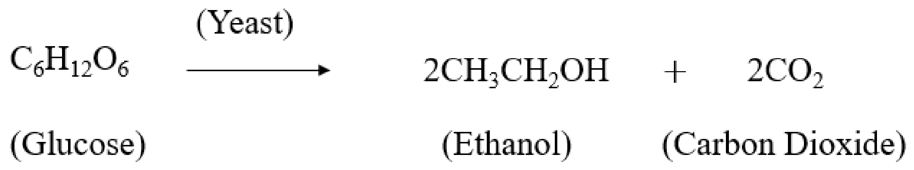
Another example of closed system are the fermenters; however, they are aimed for heterotrophic microalgae. Fermenters can reach high altitudes and are not limited by a land extension. Fermenters share the benefits of the photobioreactors of a more controlled environment with less or no pollution. Fermenters' complication is the one related to the heterotrophic algae, which is the cost of the carbon source, such as sugar.

2.3.4. Fourth generation biodiesel

The fourth generation of biodiesel is very similar to the third generation with only one major difference. In the latest generation the algae used for the biofuel production are genetically modified to increase oil yield and enhance CO_2 sequestration. With the genetic modification there have been reports of oil content up to 70% of the dry biomass (Moravvej *et al*., 2019).

2.4 Bioethanol

Bioethanol is produced through the conversion of sugars into alcohol. The conversion is done through alcoholic fermentation carried out by microorganisms such as *Saccharomyces cerevisiae* (Figure 4). "*With its high octane number of 108, bioethanol becomes a favourable fuel internal combustion engine to prevent engine knocking and early ignition, thus leads to high antiknock value. Although it has 68% lower energy content compared to petrol, bioethanol's high oxygen content makes the combustion cleaner and results lower emission of toxic substances*." (Aditiya *et al*., 2016). Bioethanol could be used in regular gasoline combustion engines using gasoline-bioethanol blend. As in the case for biodiesel, there are four biofuel generations.



$$\underset{\text{(Glucose)}}{C_6H_{12}O_6} \xrightarrow{\text{(Yeast)}} \underset{\text{(Ethanol)}}{2CH_3CH_2OH} + \underset{\text{(Carbon Dioxide)}}{2CO_2}$$

Figure 4. Alcoholic fermentation.

2.4.1. First generation bioethanol

First generation bioethanol comes from edible crops such as sugarcane, sorghum, wheat, or corn. The overall procedure of first generation bioethanol production consists of four steps. The first step is the cleaning of the raw crop to select the parts of the plant or grain that can be used for the process. The second step is the extraction of sugar, where the sugar is concentrated in a juice. The third step is the fermentation where the sugars are converted into alcohol. The fourth step is the bioethanol purification where, through distillation and dehydration, the quality of bioethanol is increased. With this bioethanol generation the debate of food vs. fuel is present.

2.4.2. Second generation bioethanol

Second generation bioethanol uses "*lignocellulosic biomass as their feedstock, but can also rely on the use of industrial waste products such as crude glycerol and whey.*" (Ganguly *et al*., 2021). For the second generation bioethanol production there are two main pathways. The first pathway is the biochemical fermentation previously mentioned. The second pathway involves thermochemical conversion, in which syngas is produced from the biomass and then through the Fischer-Tropsch process ethanol is regenerated. For lignocellulosic biomass further pretreatment is needed to break the lignin without jeopardizing the cellulose. The operation costs of second generation bioethanol tend to be lower compared to the operation costs reported for the first generation bioethanol. This decrease in the operation costs is mainly due to lower feedstock costs.

2.4.3. Third generation bioethanol

Third generation bioethanol is obtained from algae biomass. Algae tend to have a high oil content, approximately 30%, but the rest of the biomass can be used for different purposes among them bioethanol obtention. Both thermochemical and biochemical fermentation can be used to obtain bioethanol.

2.4.4. Fourth generation bioethanol

Fourth generation bioethanol uses genetically engineered bacteria in the biofuel production. There are some Cyanobacteria strains that naturally ferment starch in a two-stage process. "… *cyanobacteria firstly converts pyruvate to acetaldehyde through decarboxylation, and then reduces the produced acetaldehyde to ethanol. Nevertheless, metabolically engineered cyanobacteria are able to directly produce ethanol from carbon dioxide, water, and sunlight*." (Moravvej *et al*., 2019). Still, the newest bioethanol generation still is on early research stages.

2.5. Biofuel's controversies

The first and second generations of biodiesel have factors that limit their production. Regarding the first generation, the dilemma of the fuel vs. food is presented. The dilemma refers to the fact that the biomass could be destined as food instead of fuel, and in a world where a great sector of its population (~10%) faces severe hunger (World Hunger, 2018) this needs to be taken into consideration. Also, it not only the fact that the crop itself could be food, but how the soil is treated. If the crop is not rotated the soil can present erosion making it useless for the next cropping seasons and affect the ecosystem. So, even the overexploitation of land could make what it is considered a renewable source of energy to nonrenewable. What is done with edible sources and the proper use of the soils can be considered the two main issues of this type of biodiesel.

In the United States the production of biofuels contributed to an economic crisis. The basket of food prices increased over 100% from 2002 to 2008. "*...biofuels have been responsible for a 75% jump over that period*." (Chakrabortty, 2008). As a response the government changed its approach and gave less subsidiaries to produce first-generation biodiesel. So, biofuels production and the original policies affected a large section of the American society.

While the second generation biodiesel does not face these inconveniences, there are others that come with this type of sources. One could be that pretreatment of the oil is needed, since it is mainly waste it has a lower quality than unused oils, water and suspended solids could be present. In addition, it is limited to the consumption and waste patterns.

Furthermore, there are situations that could affect both generations. As an engineering projects it is important to analyze their efficiency and if they can obtain profit. This to see if the project is actually "generating" energy or just consuming more, and to see if it can be first of all an economic possible project and if it would be considered sustainable.

Also, a parameter to measure energy projects efficiency or value is the Net Energy Balance (NEB), also known as Net Energy Ratio (NER). This is many units of energy are produces per unit of energy consumed. The bigger the NEB the better the project. The correct measurement should consider the energy input since the beginning of the process, in this case the cultivation for first-generation biodiesel and, probably, the transportation for the second-generation. Even though the balance could vary from one project to another, the results do not represent a competitive NEB (Randelli, 2008).

There has been a small number of cases in which it represents a less expensive option than petroldiesel. Therefore, a more efficient process is looked. The use of land is a key factor in both types of biodiesel. "*Biofuel production from food crops grown in farmland will affect food security and prices, while the cultivation of nonfood energy crops will result in competition with food crops for farmland*." (Zhu *et al*., 2016). While it has advantages in comparison to the conventional diesel and fossil fuels, there are factors that still affect the profit from these projects and that question whether it can be considered a renewable and sustainable source.

2.6. Constructed wetlands

2.6.1. Application and benefits

The United States Environmental Protection Agency or EPA (2017) defines the constructed wetlands as "… *treatment systems that use natural processes involving wetland vegetation, soils, and their associated microbial assemblages to improve water quality*." Constructed wetlands use an engineering approach to take advantage of different physicochemical or biochemical processes to treat the water (Hoffmann *et al*., 2011). Constructed wetlands usually serve as a secondary or tertiary water treatment (Alarcón Herrera *et al*., 2018).

Artificial wetlands treating systems have some advantages that make them suitable for countries in development. First, they require less initial investment in comparison to other treatment methods. In addition, they are easy to build and require less technology than other process. Furthermore, the operation of constructed wetlands is cheap and relatively easy. And finally, artificial wetlands produce few sludges, having less waste than more conventional methods (Mota Torquero, 2011).

While the constructed wetlands are a technology that has been used around Europe and North America over the past decade, Latin American countries have not taken advantage of them at the same level. Some of the Latin American regions would have optimum weather conditions for the implementation of constructed wetlands on wastewater treatment trains (Alarcón Herrera *et al*., 2018).

2.6.2. UDLAP's constructed wetland

The *Universidad de las Américas Puebla* (UDLAP), as part of its efforts to improve the quality of the discharge water uses a constructed wetland as a tertiary water treatment process. The water that exits the activated sludge treatment plant goes into the wetland. The UDLAP's artificial wetland was built on 2010 and has been operating ever since. Figure 5 represents the constructed wetland.

Figure 5. UDLAP's constructed wetland diagram (Mata Toquero, 2011).

UDLAP's wetland has an area of 400 m^2 and a depth of 1.5 m. To avoid infiltration the excavation was covered with a geomembrane 1 mm thick. During its initial stage two plant species were introduced. The first specie was *Typha dominguensis* known as *Tule* in

Spanish, 10 of these plants were included. And the second specie consisted of *Lemna minor* or duck weed, commonly called *lenteja de agua* in Spanish, 100 kg in wet basis were introduced into the wetland. While the *Lemna minor* population has remained relatively constant until now, the *Typha dominguensis* population has decreased to almost 2 plants based on observations made in 2020 as shown on Figure 6.

Figure 6. UDLAP's artificial wetland current state.

For UDLAP's constructed wetland the maintenance consists of the removal of the duckweed before its life cycle ends. It was found that once its life cycle ends, the organic material sinks and eventually decomposes generating methane and carbon dioxide. Up to date, in the UDLAP there is no particular use of the extracted *Lemna*. Also, there is no specific maintenance schedule, but the removal is done based on the visual observations to the wetland.

2.7. *Lemna minor*

The common duckweed, *Lenteja de Agua*, comes from the genus *Lemna* and is found in open ponds, wetlands, or similar structures. "*Individual plants consist of a single, flat oval leaf* *(technically a modified stem) no more than ¼ of an inch long that floats on the surface of still-moving ponds, lakes, and sloughs*" (Fertig, n/d). *Lemna minor* (Figure 7) are relatively small plants, being 2-4 mm in length and 2 mm thick (Arroyave, 2004).

Figure 7. Lemna minor (Naturalista, 2020).

2.7.1. Habitat

The *Lemna minor* is a plant that can be found on different places around the world. It can be seen in America, Asia, Europe, Australia, and New Zealand (Arroyave, 2004). The United States Department of Agriculture through its Forest Service indicates that this duckweed is found in all states excluding Hawaii and South Carolina. (Fertig, n/d). While Mexico does not have an official record of the locations of this plant, observations have been reported (Figure 8) on states like Puebla, Veracruz, Sinaloa, among others through Naturalista. Naturalista is a platform where users can upload and report observations of different plants or animals in collaboration with the *Comisión Nacional para el Conocimiento y Uso de la Biodiversidad* (CONABIO) or National Commission for the Knowledge and Use of Biodiversity (Naturalista, 2020).

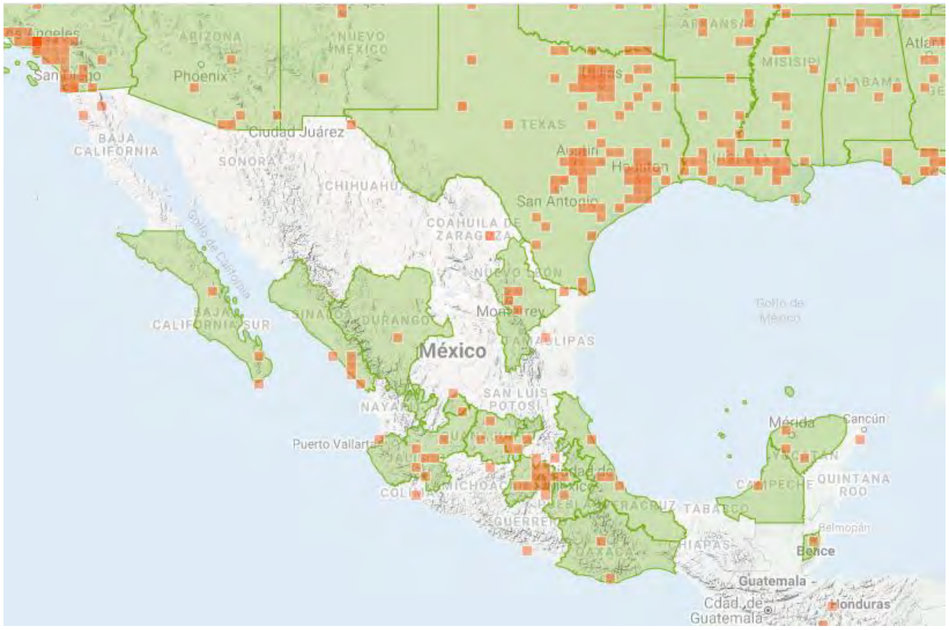


Figure 8. Lemna minor's reports in Mexico (Naturalista, 2020).

Leman minor grows on template and tropical conditions. While the duckweed can develop on temperatures between 5°C and 30°C, it thrives on a range between 15°C and 18°C. *Lemna minor* can also withstand a wide pH range, from 4.5 to 7.5 (Arroyave, 2004). This shows that they can be grown on different places within Mexico.

2.7.2. Conventional uses

The duckweed has been proven useful for different application. There are three common uses for these plants. The first one is as feedstock for animals. The second one in water treatment processes or phytoremediation. And the third one as a toxicity test.

Its use as animal feedstock would be the first and most common use. Since the duckweed has a high protein content it is very suitable as animal food. In Mexico it has been used to feed pigs, which could reduce the soy protein consumption by 80%. As its common name refers, it has also been used to feed domestic ducks, and by doing so feeding costs were reduced up to 25% in comparison to commercial feedstock (Arroyave, 2004).

As water treatment methods, the *Lemna minor* has been used in open ponds or constructed wetlands mainly for the nutrient's removal. They serve as a secondary or tertiary treatment process and the constructed wetlands also work as a good water reservoir. On a lab scale experiment, it was found that the phosphates dropped from an initial concentration of 15 mg/l to 0.5 mg/l in 8 days (Arroyave, 2004). Another study showed that it could remove 95.1% of an initial concentration of 495.3 mg/l of NO_3 in 27 days, and 100% of an initial concentration of 54.1 mg/l of NH_4 in 18 days (Ge *et al.*, 2012).

3. Methodology

3.1. Materials

For the bibliographic research and calculations of this project a computer was used with the following characteristics:

- Intel Core i5 8th Gen.
- RAM 12 GB.
- Internet connection.

Software from Microsoft Office family was used. For the calculations and most of the figures the spreadsheet Microsoft Excel. For the block diagram Microsoft Visio was used.

3.2. Methods

3.2.1. Bibliographic research

A bibliographic research was conducted to obtain the biofuel potential of the *Lemna minor*. For the research only peer-reviewed publications were accepted and search engines such as Google Scholar, ScienceDirect or Scielo were used. The first stage of the bibliographic research parameters focused on the growth rate, lipid content and biofuel potential of the duckweed. The keywords for the research were: *Lemna minor*, Duckweed, lenteja de agua, growth rate, biofuel, bioethanol, lipid content, oil content, growth, biodiesel, bioenergy. Different combinations of the keywords were used, and the research was done both in Spanish and English to increase the possible publications. To select a publication for further review the abstract had to contain one of the key words refereeing to the duckweed and at least another one refereeing to the parameter.

The second stage for bibliographic research focused on the economic, environmental, and social effects of bioenergy projects. The previously mentioned search engines and

Google, and besides peer-reviewed sources also documents from USA's government, European Union or the Mexican government were considered. The keywords for the second stage included: bioethanol production, biodiesel production, cost, investment, global warming potential, LCA, job generation. For this stage since there were no study that reflected the economic, environmental, or social impacts of *Lemna minor* bioenergy project the documents that were selected were metanalyses, reviews or compounds that included different biofuel projects or generations.

3.2.2. Calculations and estimations

Three main steps were followed to obtain the production capacity and performance indicators (Figure 9). The first step consisted of obtaining key average characteristics from the retrieved information concerning duckweed. On the second step based on the UDLAP's constructed wetland area three production scenarios were obtained, a minimum, average and maximum. Finally, with the average scenario, some environmental, social and economic indicators were obtained. A set of equations was proposed for the obtention of the indicators (see Section 4.1.)

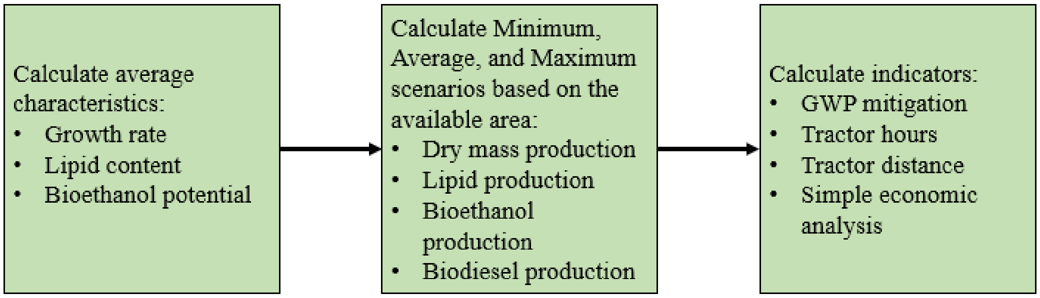


Figure 9. Calculations' block diagram.

4. Results and discussion

4.1. Performance indicators equations

After the information was gathered some estimations and calculations were required to evaluate the biofuel potential and impact of *Lemna minor* biorefinery. The first step was to obtain the average growth rate using the Equation 1.

$$G_{av} = \sum_{1}^{N} \frac{G_i}{N}$$

Equation 1. Lemna minor’s average Growth rate.

Where G represents the growth rate (g/(m^2*d)), N the number of data points. The subindexes Av and i, represent the average value and the value from the different source and conditions.

The different scenarios of *Lemna minor* production per year production were calculated using Equations 2-4.

$$M_{Min} = G_{Min} * \frac{360\ day}{1\ year} * \frac{1\ kg}{1000\ g} * A$$

Equation 2. Minimum Lemna minor's dry biomass production per year.

$$M_{Max} = G_{Max} * \frac{360\ day}{1\ year} * \frac{1kg}{1000\ g} * A$$

Equation 3. Maximum Lemna minor's dry biomass production per year.

$$M_{Av} = G_{Av} \frac{360\ day}{1\ year} * \frac{1kg}{1000\ g} * A$$

Equation 4. Average Lemna minor's dry biomass production per year.

Where M represents the yearly *Lemna minor*’s dry biomass production in one year (kg/year), and A the wetland surface area in which the *Lemna minor* grows (m^2) and for

UDLAP's case A is equal to 400 m^2. The remaining factors are to have the wanted units. The subindexes Av, Min and Max, represent the average, minimum, and the maximum, respectively.

The average oil content was obtained with Equation 5.

$$LC_{Av} = \sum_{1}^{N} \frac{LC_i}{N}$$

Equation 5. Lemna minor's average oil content.

Where LC represents the lipid content (%), N the number of data points. The subindexes av and i, represent the average value and the value from the different source and conditions.

To compare the bioethanol potential reported by the bibliography the same units were needed. It was selected to represent the bioethanol potential in volumetric unit of bioethanol production (l) per area of *Lemna minor* crop (m^2). The average value of bioethanol potential was calculated using Equation 6.

$$Be_{Av} = \sum_{1}^{N} \frac{Be_i}{N}$$

Equation 6. Lemna minor's average bioethanol potential.

Where Be represents the bioethanol potential (l/ m^2), N the number of data points. The subindexes Av and i, represent the average value and the value from the different source and conditions, respectively.

Afterwards the estimated bioethanol and biodiesel production were calculated. For each biofuel three scenarios were calculated. The first scenario represented the worst-case scenario. The second scenario represented the best-case scenario. And the third scenario represented the average scenario.

The different scenarios of bioethanol production per year production were calculated using Equations 7-9.

$$BeP_{Min} = Be_{Min} * A$$

Equation 7. Minimum volumetric bioethanol production per year.

$$BeP_{Max} = Be_{Max} * A$$

Equation 8. Maximum volumetric bioethanol production per year

$$BeP_{Av} = Be_{Av} * A$$

Equation 9. Average volumetric bioethanol production per year

Where BeP represents the yearly volumetric bioethanol production (l/year), Be the bioethanol potential (l/ m^2) and A the wetland surface area in which the *Lemna minor* grows (m^2) and for UDLAP's case A is equal to 400 m^2. The subindexes Av, Min and Max, represent the average, minimum, and the maximum, respectively.

The different scenarios of lipid obtention per year production were calculated using Equations 10-12.

$$L_{Min} = \frac{LC_{Min} * M_{Min}}{100}$$

Equation 10. Minimum lipid obtention per year.

$$L_{Max} = \frac{LC_{Max} * M_{Max}}{100}$$

Equation 11. Maximum lipid obtention per year.

$$L_{Av} = \frac{LC_{Av} * M_{Av}}{100}$$

Equation 12. Average lipid obtention per year.

Where L represents the lipid production per year (kg/year), LC the lipid content (%). And the factor $\frac{1}{100}$ is to eliminate the percentage. The subindexes Av, Min and Max, represent the average, minimum, and the maximum, respectively.

To obtain the biodiesel production the data obtained from Ordaz Santamaría (2011) was used. Three scenarios were obtained for the biodiesel production with Equations 13-15.

$$BdP_{Min} = L_{Min} * 0.963 \frac{kg_{Bd}}{kg_L} * \rho_{Bd}$$

Equation 13. Minimum biodiesel production per year.

$$BdP_{Max} = L_{Max} * 0.963 \frac{kg_{Bd}}{kg_L} * \rho_{Bd}$$

Equation 14. Maximum biodiesel production per year.

$$BdP = L_{Av} * 0.963 \frac{kg_{Bd}}{kg_L} * \rho_{Bd}$$

Equation 15. Average biodiesel production per year.

Where BdP represents the biodiesel production per year (l/year), 0.963 is the ratio between biodiesel:lipid (kg of biodiesel/kg of lipid) Ordaz Santamaría (2011). And ρ_{Bd} is the biodiesel density 0.880 kg/l. The subindexes av, min and max, represent the average, minimum, and the maximum, respectively.

The power generated $\dot{W}$ (kW) was calculated with the Equations 16-18.

$$\dot{W_{S1}} = BeP_{av} * w_{Be} * \frac{1\,h}{3600\,s} * \frac{1\,d}{24\,h} * \frac{1\,year}{360\,d} * \frac{1000\,kW}{1\,MW}$$

Equation 16. Power generation of section 1.

$$\dot{W}_{S2} = BdP_{av} * w_{Bd} * \frac{1\,h}{3600\,s} * \frac{1\,d}{24\,h} * \frac{1\,year}{360\,d} * \frac{1000\,kW}{1\,MW}$$

Equation 17. Power generation of section 2.

$$\dot{W}_T = \dot{W}_{S1} + \dot{W}_{S2}$$

Equation 18. Total power generation.

Where w represents the energy density (MJ/l) with a value of 21.10 of bioethanol and 33.30 for biodiesel. The other factors are to match units. The subindexes $S1$, $S2$, Bd, Be, and T represent the average, section 1, section 2, biodiesel, bioethanol, and total, respectively.

To obtain the costs of the project both capital (CAPEX) and operational costs (OPEX) were divided into two sections. The first section would consider the costs related to the bioethanol production. And the second section would consider the costs related to the biodiesel production. Both CAPEX and OPEX were obtained with data from the European Commission (Kalligeros e*t al.*, 2018). For the two sections the CAPEX was calculated with Equations 19 and 20.

$$CAPEX_{S1} = BeP_{Av} * C_{Be}$$

Equation 19. Capital costs section 1.

Where $CAPEX_{S1}$is the Capital cost of the section 1 of the plant (MXN), and C the capital cost required per unit of ethanol (MXN/l) with a value of 58.12 MXN/l (10 USD/gal) (Kalligeros e*t al.*, 2018).

$$CAPEX_{S2} = \dot{W}_{S2} * C_{Bd}$$

Equation 20. Capital cost section 2.

Where $CAPEX_{S2}$ is the Capital cost of the section 2 of the plant (MXN), and C the capital cost required per unit of power produced (MXN/kW) with a value of 18,000

MXN/kW (750 EUR/kW) (Kalligeros e*t al.*, 2018). For the total CAPEX was calculated with Equation 21.

$$CAPEX_T = CAPEX_{S1} + CAPEX_{S2}$$

Equation 21. Total capital costs.

Operation and maintenance expenses were also calculated with information from the European Commission (Kalligeros e*t al.*, 2018). For the two sections the OPEX were calculated with Equations 22 and 23.

$$OPEX_{S1} = \dot{W}_{S1} * O_{Be}$$

Equation 22. Operational costs section 1.

Where $OPEX_{S1}$is the operation costs of the section 1 of the plant (MXN/year), and O the operational and maintenance cost required per unit of power generated (MXN/kW) with a value of 3792 MXN/kW (158 MXN/kW) (Kalligeros e*t al.*, 2018).

$$OPEX_{S2} = \dot{W}_{S2} * O_{Bd}$$

Equation 23. Operational costs section 2.

Where $OPEX_{S2}$ is the operational cost of the section 2 of the plant (MXN/year), and O the capital cost required per unit of power produced (MXN/kW) with a value of 2,496 MXN/kW (104 EUR/kW) (Kalligeros e*t al.*, 2018). The total OPEX was calculated with Equation 24.

$$OPEX_T = OPEX_{S1} + OPEX_{S2}$$

Equation 24. Total operational costs.

The savings of the project were calculated with Equations 25-27. Where S is the savings per year (MXN/year), u is the unit cost of the fuel that will be avoided (MXN/l). 8 MXN/l for bioethanol and 20 MXN/l for biodiesel. The subindexes Av, Be, Bd, $S1$, $S2$, and T represent the average, bioethanol, biodiesel, section 1, section 2, and total, respectively.

$$S_{S1} = BeP_{Av} * u_{Be}$$

Equation 25. Savings section 1.

$$S_{S2} = BdP_{Av} * u_{Bd}$$

Equation 26. Savings section 2.

$$S_T = S_{S2} + S_{S1}$$

Equation 27. Total savings.

For the global warming potential (GWP) mitigation the Equations 28-29 were used. Data from the review on different biofuel generation from Carneiro and colleagues (2017) was used.

$$GWP_{M,be} = E_E - E_{Be}$$

Equation 28. Global warming potential mitigation per unit power from bioethanol.

$$GWP_{M,bd} = E_D - E_{Bd}$$

Equation 29. Global warming potential mitigation per unit of power from biodiesel.

Where GWP_M is the global warming potential mitigation (g $CO_{2\ eq}$/MJ), E the greenhouse gas emissions (g $CO_{2\ eq}$/MJ). The subindexes E, D, Be, Bd, and M represent the ethanol, diesel, bioethanol, biodiesel, and mitigation, respectively. The used values of GHG emissions used are on the Table 1.

Table 1. Greenhouse gas emissions of different fuels (Based on Carneriro et al., 2017).

Fuel	**E_i, g $CO_{2\ eq}$/MJ**
Ethanol	91.9
Diesel	88.6
Bioethanol	32
Biodiesel	34

The GWP mitigation per year was obtained with Equations 30-32

$$GW\dot{P}_{M,S1} = GWP_{M,Be} * BeP_{Av} * w_{Be} * \frac{1\ ton}{1000000\ g}$$

Equation 30. Global warming potential mitigation from bioethanol production.

$$GW\dot{P}_{M,S2} = GWP_{M,Bd} * BdP_{Av} * w_{Bd} * \frac{1\ ton}{1000000\ g}$$

Equation 31. Global warming potential mitigation from biodiesel production.

$$GW\dot{P}_{M,T} = GW\dot{P}_{M,S1} + GW\dot{P}_{M,S2}$$

Equation 32. Total global warming mitigation from Lemna biorefinery.

The social impact was calculated in tractor hours (Equation 33) and the distance traveled by the tractor (Equation 34).

$$t = \frac{(BeP_{av} * w_{Be} + BdP_{av} * w_{Bd})}{T} * \frac{1\ h}{3600\ s}$$

Equation 33. Tractor hours obtained from the project.

$$d = 0.725 \frac{km}{kWh} * (BeP_{av} * w_{Be} + BdP_{av} * w_{Bd}) * \frac{1\ h}{3600\ s} * \frac{1000\ kWh}{1\ MWh}$$

Equation 34. Tractor distance obtained from the project.

Where t is the tractor hours gained in a year (h/year), T is the tractor power demand of 0.0477 (MW) (Bietresato, 2019), the other factor is to have unit consistency. On Equation 34, d stands for the distance traveled by the tractor in a year using the fuel (km/year), where the factor 0.8625 km/kWh is the average energy demand reported in Latin America for tractors (Debernardi de la Vequia *et al*., 2017).

4.2. *Lemna minor's* biofuel potential

Unlike other feedstock like corn, or *Jathropa*, there is few information available on the literature about the biofuel production from *Lemna minor*, but still there are a few works on the subject. There are examples of different biofuels produced from the duckweed.

Some of the studies have also registered *Lemna minor* growth rate. Cheng and colleagues (2002) reported a maximum growth rate close to 29 g m^{-2} day^{-1}. Another study of duckweed growth in leachate reported lower growth rate of 7.03 g m^{-2} day^{-1} and 4.87 g m^{-2} day^{-1} for synthetic and dumpsite leachate respectively (Iqbal *et al*., 2019). On the study about mass production of *Lemna minor* reported a 702.5 kg/ha/month growth rate, in dry weight (Chakrabarti *et al*., 2018). There is a wide range from 1.37 g/(m^2*d) to 49.32 g/(m^2*d). The average value found was 14.20g/(m^2*d). Most of the reported growth rates come from studies related with the swine industry. Table 2 shows the different growth rate found on the literature. It is worth mentioning, that none of the found studies have a growth rate value from Latin America.

Table 2. Duckweed growth rate at different conditions.

Duckweed's growth rate, g/(m^2*d)	Conditions	Source
28.60	Swine lagoon spring (20% dilution)	Cheng *et al*., 2002
25.00	Swine lagoon spring (25% dilution)	
21.30	Swine lagoon spring (33% dilution)	
17.60	Swine lagoon spring (50% dilution)	
15.70	Swine lagoon fall (20% dilution)	
12.70	Swine lagoon fall (25% dilution)	
13.50	Swine lagoon fall (33% dilution)	
4.30	Swine lagoon fall (50% dilution)	
20.00	"Optimum" conditions	FAO, n/d
49.32	"Optimum" conditions	
1.37	"Realistic" conditions	
5.48	"Realistic" conditions	
7.03	Synthetic leachate	Iqbal *et al*., 2019
4.87	Dumpsite leachate	Iqbal *et al*., 2019
2.34	Pond with organic manure (July-August)	Chakrabarti *et al*., 2018
10.70	Swine wastewater (August-September)	Xu et al., 2012a
12.40	Pilot-scale culture pond using diluted pig manure	Xu *et al*., 2011
3.50	Swine Lagoon wastewater	Ge *et al*., 2013
14.10	Schenk & Hildebrandt medium	Ge *et al*., 2013
14.20	Average	

Due to its starch content *Lemna minor* has been proposed as feedstock for bioethanol production. Gusain & Suthar (2007) obtained a 0.218 g of ethanol per g of dry biomass. Another reported yield for bioethanol production is 6.42 x 10^3 L ha^{-1}, which is almost 50% higher than the ones reported for maize (Xu *et al*., 2011). Table 3 indicates the bioethanol production potential per area, having an average value of 1.03 l/m^2.

Table 3. Duckweed bioethanol potential.

Duckweed **bioethanol potential, l/m^2**	**Source**
0.64	Xu *et al*., 2011
1.41	Gusain & Suthar, 2007
1.03	Average

The composition of the *Lemna minor* could change depending on its environment and nutrient availability. Chakrabarti and colleagues (2018) cultivated the duckweed using different scenarios, using organic manure and inorganic fertilizer; reporting a composition of 36.07% to 27.12% protein, 8.45% to 7.15% lipid, 21.41% to 19.42% ash and 34.07% to 46.31% carbohydrate (all % are of dry weight). On another experiment by Gusian and Suthar (2017) reported 310.27 mg of total carbohydrates, 290.90 mg of Starch and 12.60 mg of lipids, all per g of dry biomass. Another study that grew the duckweed in sewage waters reported more than 300 mg of protein per g of dry *Lemna minor* (Hanczakowsk *et al*., 1995).

As this report aims for liquid biofuels, the lipid composition is important since it can be used for biodiesel production. Gusain & Suthar (2007) made the characterization of the lipid profile and determined that it is suitable for biodiesel production through transesterification. Table 4 summarizes the different lipid percentage found on dry matter of *Lemna minor* having as average value 5.36 %.

Table 4. Duckweed lipid content.

***Lemna minor*'s lipid content, %**	**Source**
8.45	Chakrabarti *et al*., 2018
1.26	Gusain & Suthar, 2007
4.00	FAO, n/d
4.40	
8.70	Hanczakowsk *et al*., 1995
5.36	Average

Anaerobic digestion could be used to obtain this biofuel from duckweed and has been suggested by some authors. A study in Bangladesh found that 85 L/ton could be produced annually (Nahar & Sunny, 2019). Another study that used different mixtures of Duckweed (DW) and Cattle dung (CD) to maintain an optimum C/N ratio found the better yield using a 1:1 DW:CD ratio; from 10 kg of biomass, they obtained 12,070 ml of biogas in 55 days (Yadav *et al.*, 2016). A project was suggested in Tabasco that biogas production would be a way of controlling duckweed growth and estimated that 0.971138 m^3 from biogas could be produced per day, using 9.02 kg of duckweed daily (Tovilla Peralta *et al*., 2015) still, no evidence was found of the implementation of this project.

4.3. *Lemna minor* biorefinery

While *Lemna minor* has been suggested for biofuel production it has not been categorized under any of the biofuel generations. The *Lemna minor* shares traits from feedstock of both second and third generation feedstock. In the first place, the *Lemna* is not consumed by humans, so it can be considered a non-edible feedstock. In addition, it could be presented as agro-industrial waste if the *Lemna* is used in water treatment processes or as animal feedstock. However, its aquatic nature results in similarities with third generation feedstock.

Lemna does not compete with arable land, since the constructed wetlands could be placed in land that cannot be used for other crops. Still, unlike algae, since *Lemna minor* is a surface plant, photobioreactors would not be suitable. With these factors in mind, the present study proposes a process that uses the *Lemna minor* as feedstock for biodiesel and bioethanol.

The first step of the process would be to remove the water of the *Lemna minor*. The *Lemna* comes with a high percentage of water (~70%) so the water content needs to be lowered. To reduce operation costs, the drying can be done outdoors using the sun and ambient air as main drivers. However, if the ambient conditions do not allow for high humidity removal another method could be recommended. With the drying the biomass would be ready to go through the second pretreatment stage.

The second pretreatment stage consists of the oil recovery. While *Lemna minor*'s oil content tend to be lower than the ones reported for other feedstocks, up to ~9% vs. up to 30%, it can still be used to produce biodiesel. The study by Gusain & Suthar (2007) made the characterization of the oil profile from *Lemna minor* and found that it is suitable for biodiesel production. There are different oil extraction methods that could involve mechanical agents such as press filters or chemical agents such as solvents. But, since the oil content present in the duckweed is less than 10% mechanical methods that have a relative low lipid yield were not selected, and the chemical extraction was chosen.

After the oil extraction the process would be divided into the production of two biofuels. On one hand, the biomass rich in starch and cellulose will be directed to the bioethanol production referred as section 1. On the other hand, the stream with high oil content would be directed to the biodiesel production, referred as section 2.

For the biodiesel production the methodology recommended by Ordaz Santamaría (2011) would be followed, since it yielded the better results optimizing the energy consumption, economic factors, and biodiesel quality. For the transesterification, the alcohol to use would be methanol and as a catalyzer NaOH. For every 800 g of oil 561.04 g of methanol high purity and 13.61 g of NaOH are needed. The transesterification needs to be carried out at temperature of 35 °C with constant mixing for two hours to assure the contact between reactants. For every 800 g of oil 770.30 g of biodiesel and 604.35 of glycerol are expected. Then through decantation the mass separation between biodiesel and glycerol is done.

For the bioethanol production the biomass rich in starch and cellulose will be used. To increase the sugar content a saccharification should be applied. Afterwards alcoholic fermentation will be conducted by yeast such as *Saccharomyces cerevisiae*. To purify the bioethanol mass separation through a distillation column is recommended. The bottom from the distillation column would still be rich in protein and could be used as animal food. Figure 10 represents the block diagram of the biorefinery.

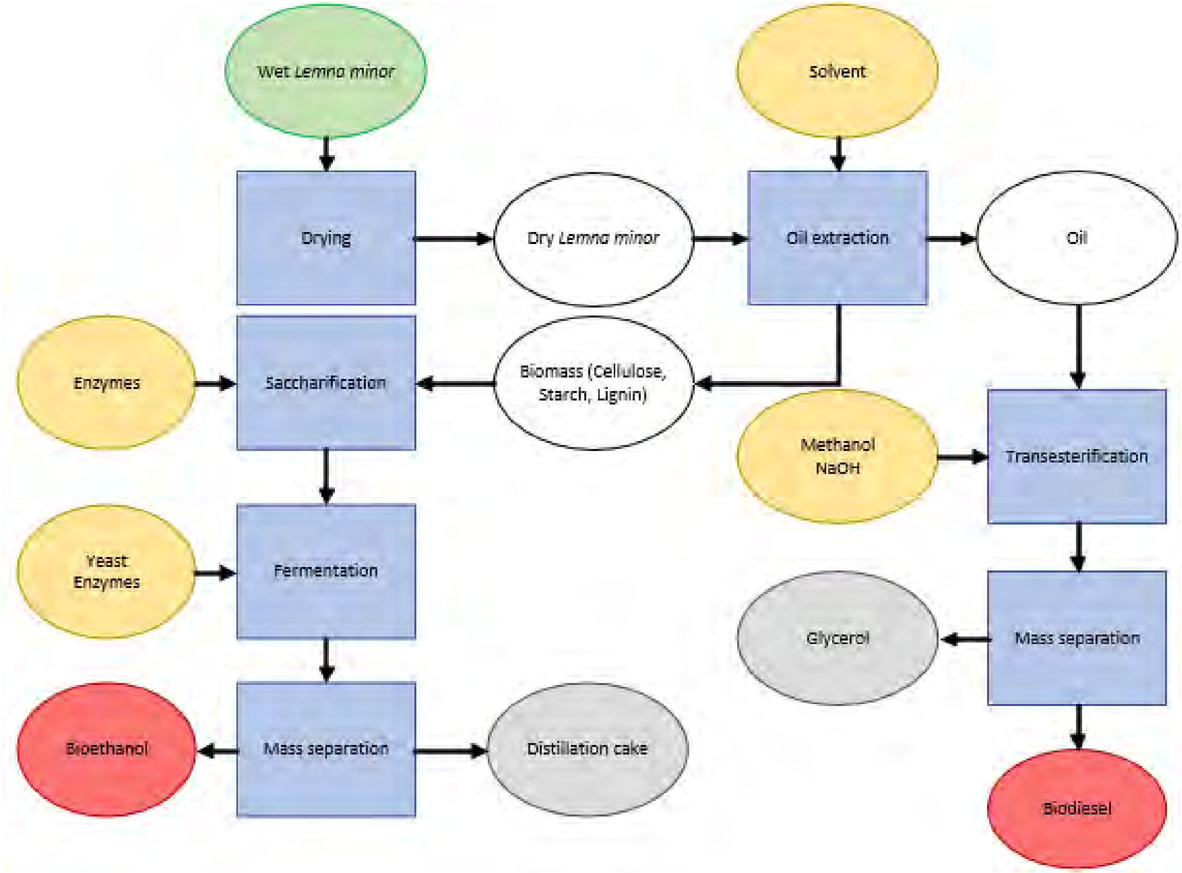


Figure 10. Lemna minor biorefinery block diagram.

4.4. Market study

While the project aims for the conceptual design of the biorefinery with a self-consumption approach for an agricultural project, to evaluate the potential market of the products in case of expansion or the development of larges projects, an individual market study was done per fuel identifying the type of industry that could buy the product, the market size, the selling price, and the trends for the following years.

4.4.1. Bioethanol and ethanol

Taking into consideration PEMEX as the sole bioethanol buyer to replace part of the MTBE in gasoline blends in Puebla. The main findings of their research are summarized in Table 5 comparing bioethanol and MTBE. One of the main findings was that bioethanol would represent a less expensive option by 8 MXN/L with higher octane. Furthermore, a benefit would be that instead of importing the MTBE from the USA, the bioethanol would come from within the country, thus supporting the local economy.

Table 5. MTBE and Bioethanol comparison.

Concept	MTBE	Bioethanol
Cost, MXN/L	14	8
Consumption in Puebla (2020), L/day	Up to 851,709.43	Up to 433,597.528 (Potential)
Octane	110	115
Place of production	United States	Mexico, United States

The possible trend for the future of bioethanol in gasoline blends would be linked to the gasoline consumption in Mexico. SENER (2013) reported the gasoline consumption in Puebla from 2002 to 2007 and made and estimation for 2013 to 2027 with a clear tendency on the rise. An estimation of the maximum potential market of bioethanol in gasoline blends was done, it is estimated as 5.6% of the estimated gasoline consumptions. This would mean that in 2020 the maximum bioethanol potential market is of 2.83 thousand barrels/day and for the 2027 of 3.538 thousand barrels/day. Figure 11 shows the reported gasoline consumption, the estimated bioethanol potential market and estimated gasoline consumption in Puebla throughout the years.

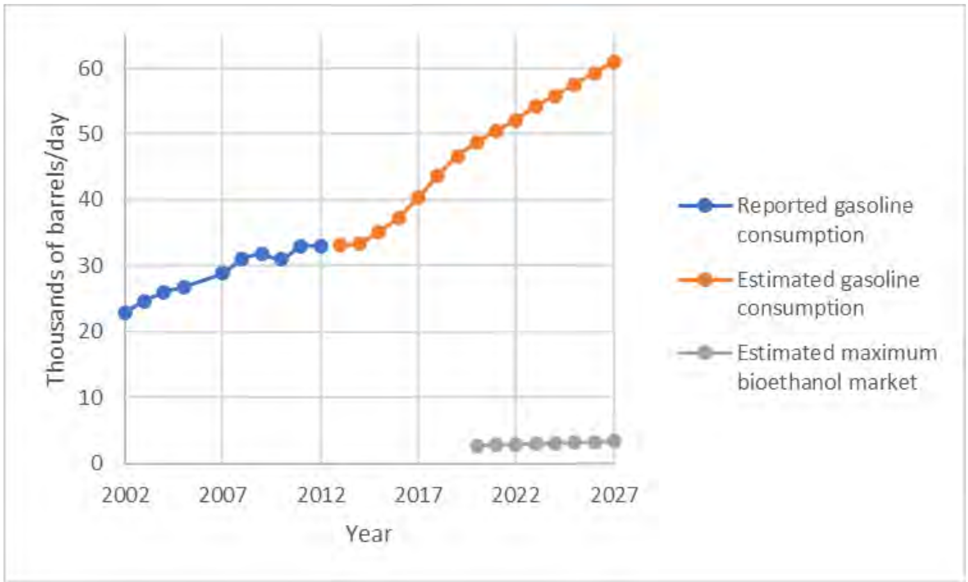


Figure 11. Puebla's gasoline consumption and bioethanol potential market in gasoline blends (Based on SENER, 2013).

Looking at a national demand, it is worth noticing that Mexico imports bioethanol from the United States. Figure 12 shows the imports from the USA over the last 7 years with data from CEDRSSA (2020). The imports have at least been 86.3 million L/year and since 2016 the trend is on the rise going from 99.5 million L to 115.5 million L.

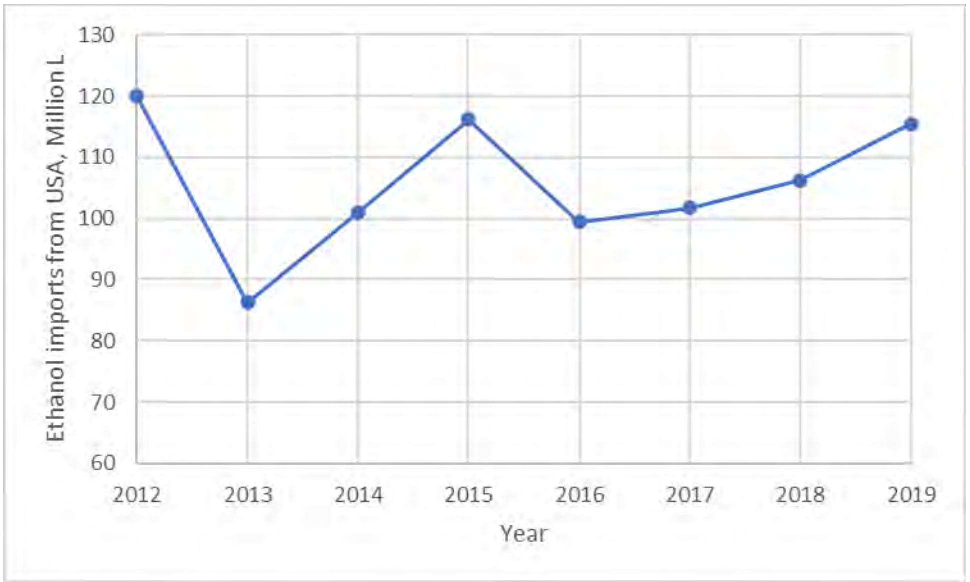


Figure 12. Bioethanol imports from the USA to Mexico from 2012 to 2019 (Based on CEDRSSA, 2020).

4.4.2. Biodiesel and diesel

When looking at the biodiesel market in Mexico there are still few biodiesel projects, but still the diesel market has grown over the years. Biodiesel could be considered as a substitute or complement of diesel, so in the present study both diesel and biodiesel are seen as part of the same market.

Riegelhaupt and colleagues (2016) report for SENER a diagnostic of the biodiesel situation in Mexico. From a total of nine biodiesel commercial scale facilities in Mexico three had stopped operations by the time the report was published. Two of the projects that have stopped operation had as a reason unavailability of feedstock. From the remaining six projects, there is a total operating capacity of 4182 m^3/year.

Regarding diesel market in Mexico the imports and prices have been raising on the last years. From 2008 to 2018 (Figure 13) the imports of diesel multiplied by a factor of more than four, going from 148.21 PJ to 658.1 PJ (SENER, 2019). While the overall diesel imports have increased, the imports from the biofuel alternative from the United States of America have decreased on the last years. Biodiesel imports from 2016 to 2019 (Figure 14) decreased from 20,440 tons to 2,240 ton (CEDRSSA, 2020). The final price of diesel to the public has increased in the last years (Figure 15) from 2008 to 2018 the price has multiplied almost by three, going from 5.13 $/l to 15.34$/l.

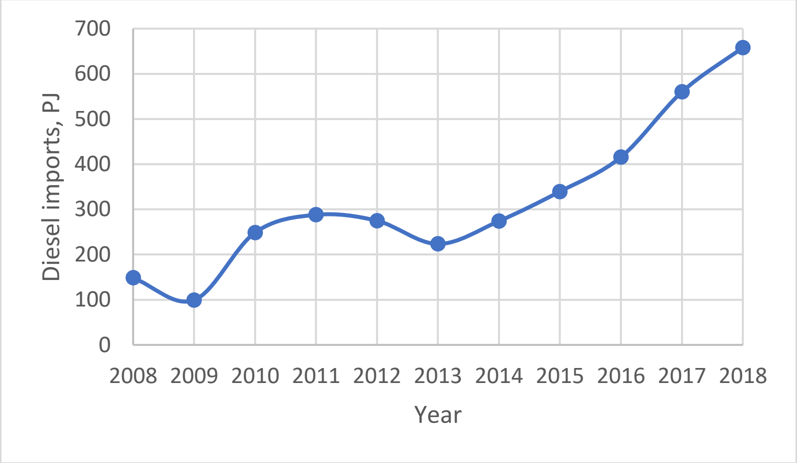


Figure 13. Diesel imports in Mexico from 2008 to 2018 (Based on SENER, 2019).

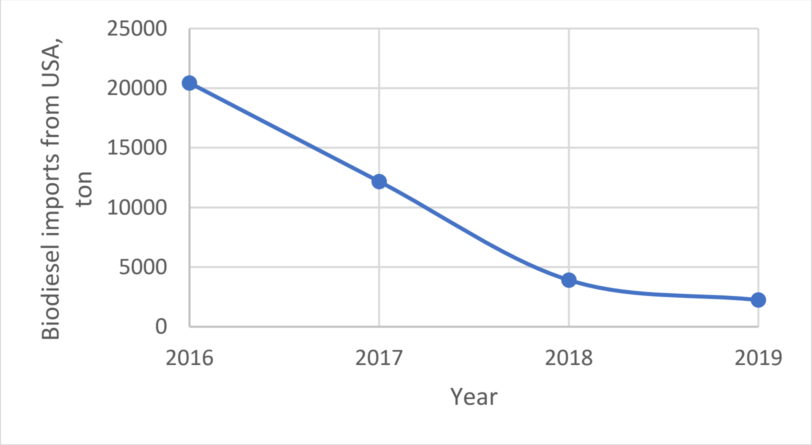


Figure 14. Biodiesel imports in Mexico from 2016 to 2019 (Based on CEDRSSA, 2020).

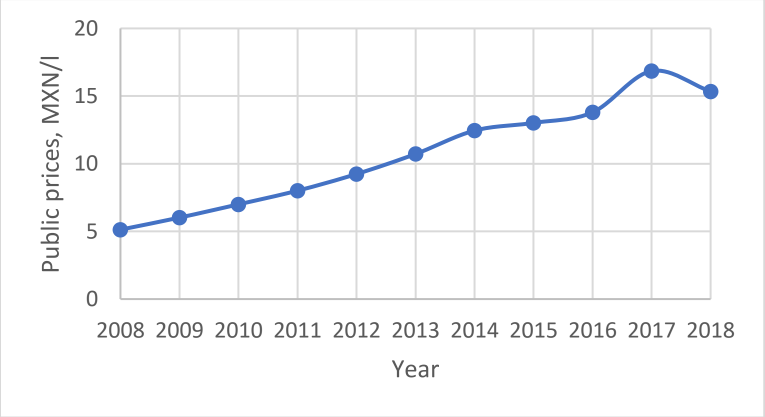


Figure 15. Diesel cost to the public in Mexico from 2008 to 2018 (Based on SENER, 2019).

4.5. Expected outcomes

4.5.1. Production

Three scenarios were predicted for the *Lema minor* dry biomass, bioethanol, lipid, and biodiesel production. The three scenarios were minimum representing the worst-case scenario, a maximum representing the best-case scenario and the average, not from the two scenarios but from the data set obtained in this project (Section 4.1). The difference between the best-case and worst-case scenarios are considerable in all four products.

¡Error! No se encuentra el origen de la referencia. shows the intermediate products (*Lemna minor* dry biomass and lipids) and final products (bioethanol and biodiesel) from biorefinery. For the expected dry biomass, the maximum and average growth rates are 36 and ~10 times higher than the minimum, respectively. The minimum variance between values is found on the bioethanol production, where the maximum and average productions are 2.2 and 1.6 times higher than the minimum, respectively. The relatively small variance could be since there are only two values on bioethanol production found on literature. On the other hand, the highest variance was found on the lipid and biodiesel production. Since the biodiesel production depends on the lipid available the variance is the same for both cases, where the maximum and average productions are 71 and 114 times higher than the minimum, respectively.

Table 6. Biorefinery intermediate and final products.

Scenario	*Lemna minor* dry biomass, kg/year	Lipid, kg/year	Bioethanol, L/year	Biodiesel, L/year
Min	197.26	2.49	256.80	2.72
Av	2,044.85	177.90	410.89	194.66
Max	7,101.37	284.05	564.99	310.81

For the project, the average values of production are used. It is considered a valid value to start the conceptual design because Puebla has optimum weather and climate conditions to make it a suitable place for the *Lemna minor*. However, the fact that the nutrient rate from UDLAP's wastewater could vary and be lower on periods with low students the maximum value could be difficult to reach, without the additions of fertilizers.

4.4.2. Environmental

Biofuel production could represent a reduction of the global warming potential (GWP) compared to the production of fossil fuels. Carneiro and colleagues (2017) made a metanalysis of different Life-Cycle-Assessment (LCA) studies to evaluate the GWP of different generations and biofuel synthesis processes. The study found a grate range of GWP, changing from one generation to another and within generations based on the process. While third generations biofuel tend to have a greater GWP based on the technological requirements of the process, for this case the average value of GWP for second generation biofuel were chosen. This value was chosen since as Xu and colleagues (2012b) have mentioned, the process would be similar to that from second-generation biofuels, both on the technology used and energy demand. Table 7 summarizes the greenhouse gas (GHG) emissions mitigated from the pilot-scale biorefinery, with a total reduction of nearly 0.9 ton of $CO_{2\,eq}$/year. So, both bioethanol and biodiesel are expected to have less GHG emissions than their fossil fuel counterpart.

Table 7. Green house potential mitigation from the project.

GHP mitigation, ton CO_{2eq}/year	
Section 1	0.52

Section 2	0.35
Total project	0.87

As mentioned on the section 2.7.2. *Lemna minor* can be used to treat water pollution. Table 8 summarizes the expected removal of pollution based on the information collected by Amare and colleagues (2018). The removal percentage presented would be for a retention time of 28 days, while the values could change with lower retention time, for P high values over 90% removal have been reported in 6 days. Also, high BOD_5 removal rate could be achieved if the treatment includes activated sludges like the one at the UDLAP's WWTP. The removal rates shown, indicate the bioremediation through *Lemna minor* is a valuable treatment method with high pollution removal specially in nutrients and biological oxygen demand (BOD).

Table 8. Expected pollutant removal rate from Lemna minor wetland.

Pollution removal, %	
P	90.00
BOD_5	91.78
SO_4	78.01
N	94.66

4.5.3. Economic

Table 9 summarizes the economic aspects of the plant. The values were obtained based on the costs reported by the European Commission (Kalligeros *et al.*, 2017) where Bioethanol has higher cost, both for CAPEX and OPEX. In the study (Kalligeros *et al.*, 2017) considered the maintenance, feedstock acquisition, reactants, and other production costs. For the project, the expected savings, would be more than three times the value of the OPEX. The savings generated through the biofuel production could allow a project to increase its self-sufficiency both on an energetic and economic aspect. The return of investment would be near the fifth year of operation (Figure 16).

Table 9. Project's economic data.

Section	CAPEX, MXN@2020	OPEX, MXN@2020/year	Savings per volume of fuel, MXN@2020/L	Savings, MXN@2020/year
Bioethanol	23,882.91	1,056.98	8.00	3,287.16
Biodiesel	3,751.18	520.16	20.00	3,893.11
Total	27,634.08	1,577.14	-	7,180.27

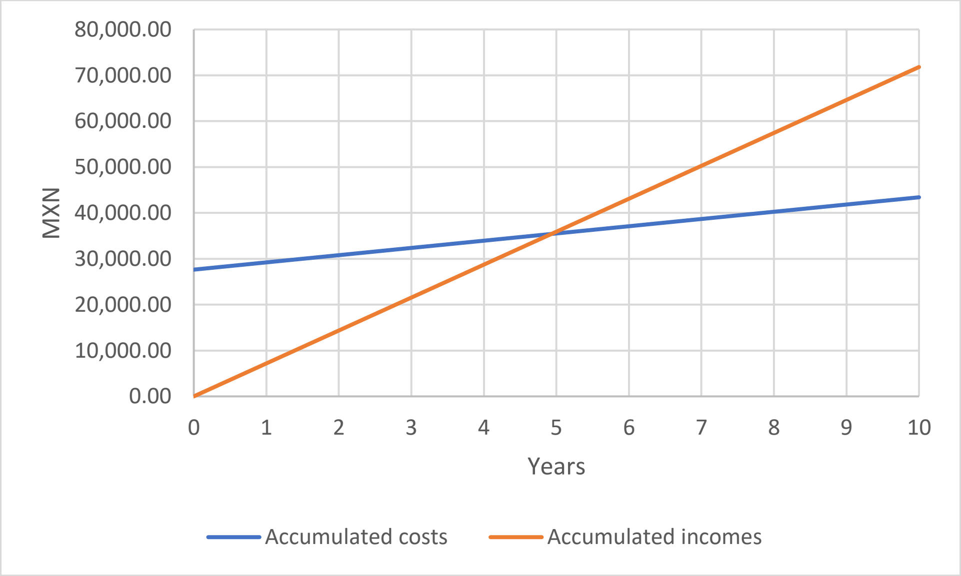


Figure 16. Accumulated gains and costs vs. Years.

4.5.5. Social

Aimed for an agricultural objective the project not only would bring economic savings but, the benefits can be translated into labor carried out by a tractor. Thus, the social impact was calculated in tractor work hours and distance obtained from the fuel production. Bietrasato and colleagues (2019) have proposed that diesel-biodiesel-bioethanol blends can be used in regular diesel tractors. The diesel-biodiesel-bioethanol blends can have better torque response in comparison to diesel-biodiesel blends (Bietrasato *et al*., 2019). To obtain the expected tractor hours and distance, it was assumed that the energy content of bioethanol and biodiesel can be added as if it were an ideal mixture and the results are shown on Table 10.

Table 10. Social benefits from the biorefinery.

Social benefits form the project	
3630.14	Tractor distance, km/year
88.24	Tractor working time, h/year

5. Conclusions and recommendations

With the present project a conceptual design of a biorefinery using *Lemna minor* as feedstock was proposed, thus fulfilling the general objective. Also, the hypothesis is proven correctly since an environmental benefit would be seen as GWP mitigation. Besides, the environmental benefit the project is profitable and technically viable.

Duckweed presents different benefits for biomass projects. The first benefit is that it does not compete with arable land since an artificial wetland can be constructed nearly everywhere. The second benefit is that it is not an edible crop by humans. And the third benefit it that duckweed can be found on almost every continent, and in Mexico there are good conditions to guarantee an optimum growth.

The present thesis aims to highlight the potential of *Lemna minor* as a feedstock for bioenergy. Its composition would make it possible to synthesize both bioethanol and biodiesel. As a response of the second specific objective, the proposed process is described in the block diagram shown in Figure 10. First a separation of the oil would be needed. Afterwards, through transesterification the oil will be converted into biodiesel and through saccharification followed by alcoholic fermentation the cellulose and starch will be transformed into bioethanol.

In relation with the third specific objective, both economic and environmental impacts were found. The project would bring global warming potential mitigation of nearly 0.9 ton CO_{2eq}/year, as the general life cycle of the biofuels is expected to have less greenhouse emissions than the respective conventional fuels counterparts. And regarding the social application, it is expected that the project brings nearly 80 tractor hours or 3,600 km in a year. The project is expected to generate around 7,000 MXN/year in savings and have a return of investments in the year five of operation. This results emphasis the benefits and potential application of the biorefinery, aiming for increasing the self-sufficiency of agricultural projects.

While the scale of the project as a pilot-plant due to the size of UDLAP's wetland, the project recognizes the potential of an up-scaling or implementation of larger projects based on the presented biorefinery. Both the ethanol and diesel consumption and imports have been growing, making room for biodiesel and bioethanol as alternatives. The generated biofuels could be used in three ways: diesel-biodiesel-bioethanol blends, diesel-biodiesel or gasoline-ethanol blends, or in engines designed for only biofuels. Also, the reporting of *Lemna minor* in different states in Mexico could mean that similar projects could be applied elsewhere. In addition, the water pollution removal capabilities of duckweed allow for the integration of wastewater treatment and energy generation processes.

There are some recommendations for future work under this line of research. A quantification of the available duckweed biomass in Mexico is still needed. Also, each project should evaluate the growth rate and composition of the duckweed to make the estimations and calculations based on the actual conditions presented. Furthermore, the link between food and energy needs to be explored deeply. While it is not a direct food for humans it is related to the cattle and swine food. The composition of the distillation cake needs to be explored to see if it is suited for animal consumption and if it would be equivalent to the raw biomass to avoid leaving the animals without food.

This project, as the first biorefinery proposed from *Lemna minor* in Mexico, aims to be the starting point for similar projects and their application. With clear environmental, economic, and social benefits bioenergy generation from duckweed could be a way of allowing agriculture to have more energetic and economic independency and applied on a larger scale could contribute to the transitioning into cleaner energy sources in Mexico.

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Annex

A1. Bioethanol potential units

Gusain & Suthar (2007) report the bioethanol potential in mass ratio as 0.22 g/g_{DM} however for the purpose of the report the wanted values were l/m^2. So, unit conversions were needed, also the average growth rate found G_{Av} was used to have unit consistency.

$$Be = 0.22 \frac{g}{g_{DM}} * \frac{(G_{Av} * 360\ d)}{\rho_{Be}}$$

Equation 35. Growth rate unit conversion.

Where it is assumed a production of 360 days, and the density of bioethanol ρ_{Be} is equal to 789 g/l.

A2. Conversions

The following conversions were used:

1 USD = 22 MXN

1 EUR = 24 MXN

1 gal = 3.785 l

1 ha = 10,000 m^2

A3. Fuel properties

The following fuel properties were used in the calculations from Sarıkoç (2019).

Table 11. Fuel properties (Based on Sarikoç, 2019)

Fuel	Biodiesel	Bioethanol
ρ, g/l	880	789
w, MJ/L	21.1	33.3