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The carbon footprint of polysaccharide production from red microalgae

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1. INTRODUCTION

Polysaccharides are present in all organisms and exhibit a great variety of biochemical structures based on glycosidically linked between monosaccharides (Cedric et al., 2016). Natural polysaccharides are gaining increased recognition as a result of the tendency of global markets to find alternatives for synthetic products to natural products and a growing understanding of the functions of sulfated sugars.

The industry of cell wall production for use in various applications has until recently, relied mostly on the seaweed colloids. For a long time, the dominant, most valuable sulfated seaweed polysaccharides in industrial applications- use largely as stabilizers and thickness- have been agar-agar and carrageenans.

However, increasing demand for these colloids in addition to resource instability (due to intensive harvesting and ecological damage) has led this industry to search for alternative, sustainable sources for algal polysaccharides. Red microalgae have been proposed as one such alternative source.

The increasing market demand for natural polysaccharides for the food, cosmetics and pharmaceutical industries cannot be met by currently available conventional sources- red and brown macroalgae. These traditional sources of polysaccharides, which are usually harvested from their natural habitats, are being depleted by intensive harvesting and detrimental environmental conditions. An attractive alternative may be found in the sulfated polysaccharides of the red microalgae (Arad and Levy-Ontman, 2010).

The annual market for seaweed polysaccharides is around 9,600 tons for agars, 26,500 tons for alginate and 50,000 tons for carrageenans for a market value around 1 billion US dollar (Cedric et al., 2016).

The annual world production of microalgae by industry is estimated to be between 10,000 and 20,000 tons for a global market between 0.5 and 5 billion euros, and this market is still developing (Cedric et al., 2016).

1.1 Algae

Algae are photosynthetic organisms that develop in varied habitats, mostly in aquatic environments. The algae are capable of converting light energy and carbon sources, such as carbon dioxide, into “biomass”.

Depending on their size, they can be classified into two broad categories: “macroalgae” and “microalgae”. Macroalgae are multicellular algae of around one centimeter in size which usually grow in ponds of natural fresh water or salt water, the largest of them are the seaweeds. Microalgae have a size measured in micrometers and considered to be single cell algae which grow in suspension, mainly in aqueous solutions (Sihem et al., 1988).

1.2 Red Microalgae

The red microalgae belong to the *Rhodophyta* division, order *Porphyridiales*, the familiar are *Porphyridium* and *Rhodella*. The reproduction of the red microalgae is asexual and their cultures have a fast growth rate. Presence of accessory pigments determines the color of the red microalgae. Some of the strains grow in fresh water and others in seawater, unicells or colonies form (Taylor et al., 1999).

1.3 Red microalgal Sulfated cell wall polysaccharides

Polysaccharides play important roles in a variety of functions in the cells of different organisms. In the red microalgae the cell walls are encapsulated within a sulfated polysaccharide in a form of a gel. During growth in a liquid medium, the external part of the polysaccharide undergoes dissolution from the cell surface into the medium (soluble fraction), whereas most of the polysaccharide remains attached to the cell (bound fraction). The gel structure of the polysaccharide in the red microalgae protects the cells from environmental extremes due to its stability to temperature, PH and salinity (Arad and Levy-Ontman, 2010).

The studies that have been performed on red microalgae have been devoted mainly to four species, the Soluble polysaccharide of those species has a common structural feature galactan heteropolymers with molar mass of $2\text{-}7\cdot10^6$ Da and contain sulfate residues.

The main sugars in the polysaccharide of the studied species are glucose, xylose and galactose in different ratios with additional minor sugars. The precise structure of the polysaccharides of the red microalgal cell wall is not fully understood due to their complexity and the lack of known enzymes that degrade them.

The polysaccharides are anionic owing to the presence of GlcA and half ester sulfate groups.

The polysaccharides have different sulfate contents 1-9% w/w. The highest antiviral activity was found in the polysaccharide having the highest sulfate content.

One of the main characteristics of the red microalgal polysaccharides that makes them suitable for industrial applications is their fluid-dynamic behavior- highly viscous aqueous solutions at relatively low polymer concentrations, yielding rheological properties comparable with industrial polysaccharides.

The red microalgal polysaccharides exhibit various bioactivities that have nutritional, medicinal and cosmetic significance.

The polysaccharides act as dietary fibers and can be valuable in a functional food.

The cell wall sulfated polysaccharide was shown to have antitumor, antiviral, anti-inflammatory, Anti-irritating and antioxidant activities.

Owing to these bioactivities, this sulfated polysaccharide has already been introduced into a wide Range of cosmetics products.

1.4 The process

The process divided into 5 main stages: Preparation and sterilization (autoclave) of the growth medium, Cultivation of the red microalgae in the bioreactor, harvesting of microalgae and separation between biomass and the solution of the polysaccharide (centrifuge), desalting of the solution (ultrafiltration) and Dry freezing of the solution in order to achieve concentrated polysaccharide (lyophilization).

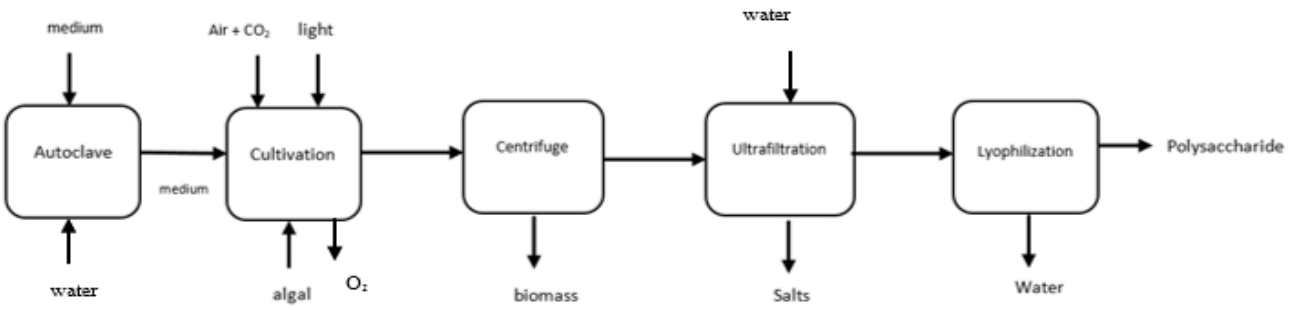

Figure 1: General overview of the production of polysaccharide from red microalgae

1.4.1 Preparation and sterilization of the medium

The cultivation of the microalgae can be conducted on varied of culture medium, depends on the species of the microalgae. The media contain nutritional elements (nitrogen, sulphate, trace metal, vitamins etc.). The species of red microalgae used on this study grow in marine water, therefore an artificial seawater has been prepared for the cultivation (Jones et al., 1963):
The main salts: 27.0 g/l NaCl, 6.6 g/l $MgSO_4 \bullet 7H_2O$,5.6 g/l $MgCl_2 \bullet 6H_2O$, 1.5g/l $CaCl_2 \bullet 2H_2O$, 1.0 g/lKNO_3, 0.07 g/l KH_2PO_4, 0.04 g/l $NaHCO_3$.
20 ml/l 1M Tris-HCl PH 7.6 (0.8 M Tris-HCl 0.2 M Tris-base)
1ml/l trace metal solution: 4mg $ZnCl_2$, 60mg H_3BO_3, 1.5mg $CoCl_2 \times 6H_2O$, 4mg $CuCl_2 \times 2H_2O$, 40mg$MnCl_2 \times 4H_2O$, 37mg $(NH_4)_6Mo_7O_{24} \times 4H_2O$ dissolved in 100ml distilled water
1ml/l of chelated iron solution: 240mg $FeCl_3 \times 4H_2O$ per 100ml 0.05M Na_2EDTA, PH 7.6In order to complete the medium volume, DDW is required.
Stock cultures were maintained in the above medium plus 1.5% Bacto Agar.
After the media preparation, the media is sterilized from microorganisms by the autoclave device.

Autoclave

Autoclaves are devices that use steam under pressure to sterilized items such as surgical instruments, solutions and materials that used in microbiology (Barbara et al., 2000).
After the sterilization, the algae have been added to the medium.

1.4.2 Cultivation

The culture conditions that are necessary for the growth of the red microalgae are:
Light- that can be provided by artificial light, fluorescent lamps, or can also be grown outdoors, using natural daylight.
Nutrients- inorganic salts required for the microalgae growth.
Carbon dioxide- using carbon dioxide gas for the growth of the algae, the capability of the red microalgae of converting light energy and CO_2 into biomass.
CO_2 is supplied from compressed gas cylinders in the air supplied to the culture (Garrod et al., 1991).
Heat- the optimal temperatures for red microalgae are from 21^oC-26^oC (Golueke et al., 1961).

Mixing- the necessary light, CO_2 and nutrients become available to all of the cells by mixing, algae cultures are usually mixed by bubbling air through them.

1.4.2.1. A closed system- polyethylene sleeves

The Arad group has developed a system for cultivate red microalgae for large scale production of polysaccharide (Debasis et al., 2015).

The system is comprised of closed vertical sleeves, each one of the sleeves is a transparent tube made of polyethylene. The sleeves are sealed at one end and looped around iron tube, on the other end. Each sleeve will be filled with the culture medium and a stream of air containing CO_2 will supply to the culture. The transparency of the tube allows the light to penetrate so that will occur a process of photosynthesis (Cohen et al., 1991)

In the sleeves cultivated outdoor temperature above 28^{o}C is prevented by spraying the outer surface with water.

***Figure* 2:** A closed cultivation system of polyethylene sleeves.

1.4.2.2 Open ponds

This cultivation method is basing on shallow open ponds, stirred with paddle wheel to prevent settling of the algae and create homogenous suspension. The main system used today is open raceway that consist several units combine together (Arie et al., 1998).

The main advantages of the open ponds are their low cost and ease of construction and operation. Disadvantages of the system include contamination with unwanted species such as foreign algae, yeast, bacteria and predators, evaporation of water, diffusion of CO2 to the atmosphere and low control over environmental conditions, particularly temperature and solar irradiation (Griffiths et al., 2011).

Figure 3: cultivation in open pond, raceway system.

1.4.3 Centrifuge

Centrifuge is a device that serves to separate solid and liquid phases from a feed which may be in a form of a slurry. This physical separation technique is basing on density differences (settling principle) or particles size (filtration principle). The slurry whirled rapidly by the device, a centrifugal force is made and aids the phases separation. Centrifuges that operate on a settling principle make the denser phase brought to the outside with the help of the centrifugal force. (Judson et al., 2013). centrifuge on the process

After the cultivation of the red microalgae, there is the need to separate between the biomass and the soluble polysaccharide. The red microalgal culture is centrifuge and produce two main dietary ingredients: a pellet containing the algal cells, biomass, and the supernatant containing the solution of the polysaccharide dissolves in the medium. The supernatant fraction comprised from the soluble polysaccharide, salts and water. On the next stage the solution desalted by the ultrafiltration device.

1.4.4 Ultrafiltration

The definition of filtration is the separation of at least two components from a fluid stream based on size differences. Membrane filtration extends the application by allowing the separation of dissolved solute in liquid stream and separation of gas mixtures. The membrane acts as selective

barrier, it permits some of the components to pass and others retain (Munir et al., 1998). Ultrafiltration membrane retain solute with hydraulic diameters in the range of 5-150 nm, this range is roughly equivalent to 1-1000 kDa molecular weight. Ultrafiltration can be run in NFF (normal flow filtration) mode or in TFF (tangential flow filtration) mode. NFF mode involves passing the solvent through the filter under pressure where the fluid velocity is perpendicular to the plane of the membrane. TFF mode involves adding another fluid velocity component parallel to the plane of the membrane, it facilitates backflow of solute and prevents filter plugging (Herb et al., 2015). Diafiltration is an extension of ultrafiltration uses the addition of water at some stage during the concentration process. The role of the diafiltration is to wash out more of the lower molecular weight components (Grandison et al., 1996).

1.4.5 Lyophilization

Lyophilization or freeze drying is commonly used in the food and pharmaceutical industries. A lyophilization process consists of three phases: I) Freezing to solidify the material, II) Sublimation drying to reduce the humidity to approximately 4% w/w of the dry product, and III) Second drying to reduce bound moisture to the final value which often is below 1% w/w. After that, the polysaccharide is frozen, lyophilized and then powdered (Goldberg, 1997).

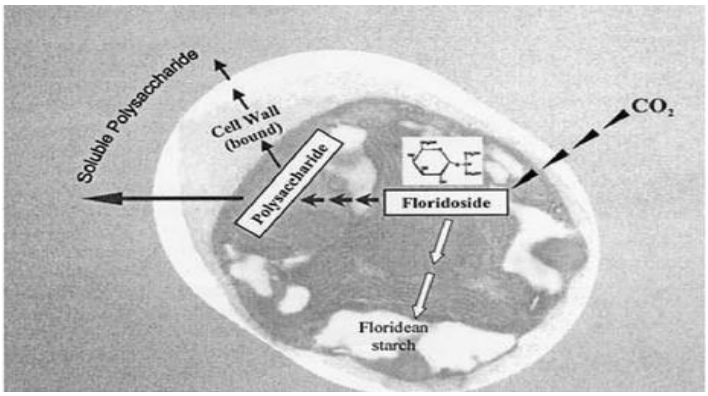

Figure 4: *dissolution of sulfated cell wall polysaccharide, red microalgae*

1.5 Life cycle assessment (ISO 14040)

The assessment of environmental impacts of a product across its life cycle.

It means that the product is followed from the level which the raw materials extracted from the natural resources until the disposal of the product (Baumann et al., 2004). Conventional impact categories to assess are: Global warming, Abiotic depletion, Acidification, Eutrophication, Ozone layer depletion, Human toxicity, Freshwater aquatic ecotoxicity, Marine aquatic ecotoxicity, Terrestrial ecotoxicity and Photochemical oxidant formation. A Life cycle assessment study is performed in four stages:

Goal and scope definition- on this phase there is a decision regarding to the product and the purpose of the study.

It includes the subject of the study, functional unit and system boundaries that have been chosen.

Inventory analysis (LCI)- creation flow model that based on the system boundaries, the model is a flowchart and includes the activities in the analysis system. The inventory analysis also contains the input and output data for all the activities and the calculations of the amount of resource use and pollutant emission relatively to the functional unit.

Impact assessment (LCIA)- the goal of this stage is to describe or indicate the impacts of the environmental loads, it means that the results from the inventory stage turn to more relevant environmental information. The first step is to classify the inventory parameters according to the contribution of each parameter to the type of the environmental impact.

the next step is to characterize, the relative contribution of all the emissions and resource consumptions to each type of impact are calculated.

Interpretation- discussion about the results and recommends.

1.5.1 Carbon footprint and GHG

Numerous phenomena have been observed over the years following the climate change, for example the global warming causes to the increasing temperature worldwide, raising of the oceans, melting ice etc. The current climate change is linked to the high emissions of greenhouse gases (GHG) produced by anthropogenic activities. Carbon footprint is a tool to measure the total amount of GHG emissions that caused directly or indirectly by company or specific product in the whole

supply chain. The main gases included in the emission calculations are typically: carbon dioxide (CO_2), methane (CH_4), nitrous oxide (N_2O), hydrofluorocarbons (HFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF6). The total amount of GHG emissions expressed by CO_2 equivalent, each gas emission transformed into CO_2 equivalents that will cause the same warming effect (Avlonas et al., 2014).

2. METHODOLOGY

2.1 Goal and scope definition

The goal of this study is to assess the carbon footprint associated of the production of 1kg of exo-polysaccharide (EPS) from red microalgae in two different systems of cultivation. The cultivation of the red microalgae considers two different systems: open ponds in pilot scale and closed system-polyethylene sleeves in lab and pilot scales. Both systems in pilot scales uses natural sun light and the lab scale uses artificial illumination. The lab experiments regarding the cultivation were carried out by Esther Lalzar- Saban (personal communication). Due to the difficulties to obtain certain figures, the data for this scale was completed by with literature data. The pilot scale experiments regarding the cultivation were determined from literature data only. There are two sources of electricity consumption that have been chosen, Israel mix grid and Photovoltaic solar energy (PV) due the crucial parameter of power consumption.

2.1.1 Scenario A-polyethylene sleeves lab scale process

Indoor cultivation of the red microalgae in polyethylene sleeves take place on a lab scale. To reach 1 kg of EPS in lab scale a reference scale was made based on the assumption that the connection between the culture volume/ number of cells to polysaccharide and biomass production is linear. The process of polysaccharide production was divided into five stages: I) Preparation and sterilization of the medium culture, II) Cultivation of the red microalgae, III) Harvesting the microalgae by centrifuge, IV) Desalting the EPS solution by ultrafiltration, and V) Dry freezing by lyophilization.

Preparation and sterilization of the culture medium

The medium contains water and nutrients according to the concentrations mentioned on the introduction. On the lab scale, a volume of 900ml culture medium was required at the beginning for the first run of the reactor. This volume was sterilized by laboratory autoclave device for 2.25 h. The reference scale contains 520 L culture medium at the beginning and sterilized by autoclave device. After the sterilization, the initial inoculum was added, on the scale lab it was 300 ml and the

reference scale kept the same ratio so it be 173 L. The next stage is the cultivation of the microalgae on the bioreactor.

Cultivation of the red microalgae

In the lab scale, volumes of 900ml culture medium and 300ml of initial inoculum were required to achieve the concentration of $4 \bullet 10^6$ cells$\bullet$ml^{-1} for the first run of the bioreactor (measurements on site). The microalgae were grown at 24 °C, 300 µmol$\bullet$m^{-2} s^{-1} light intensity from 6 fluorescent lights (each 6 W). The bioreactor consisted of 6 transparent polyethylene sleeves with capacity volume of 590ml each (diameter 5 cm and length 30 cm) and aerated by 15 L/min compressed air enriched with 1.5%-2.0% CO_2. The growth was for 38 days reaching in semi continuous mode 2.6g of soluble polysaccharide only at the cultivation stage after 5 times of harvesting. After 10 days of growth, 4.36g of dried biomass and 0.519g of soluble polysaccharide were produced (Orit et al., 1991). To reach 1 kg of polysaccharide after 38 days and 5 times of harvesting the system should be multiplied by 577 and would contain 520L culture medium and 173L initial inoculum and after 10 days of growth 2519g of dried biomass and 300g of soluble polysaccharide, which means at the end of 38 days and 5 times of harvesting would produce 1500g of soluble polysaccharide at this stage.

Harvesting

The soluble polysaccharide and the biomass produced were harvested by laboratory centrifuge with an efficiency assumption of 100%. In the semi continuous mode, the algae are cultivated and by reaching the concentration of $58.4 \bullet 10^6$ cells/ml, 65% of the total volume were harvested and diluted with pure medium when reaching $20.4 \bullet 10^6$ cells/ml (Orit et al., 1991)
The first time that a concentration of $58.4 \bullet 10^6$ cells/ml has been reached, was after 10 days of growth, the other 4 times were after 7 days each one of them from the harvesting and diluting day and 38 days of growth in total.

Ultrafiltration

The purpose of the ultrafiltration is to desalt the polysaccharide solution and it was performed with automated tangential flow filtration (TFF), membrane with normal molecular weight cut off (NMWCO) of 300KDa, surface of 15m^2 and permeability of 180 $\frac{L \cdot bar}{h \cdot m^2}$. It was performed for 100min using diafiltration technique, desalting 98% of the salt with water when the ratio between the

solution and the water is 1:4. The purification that has been reached is 0.57 $\frac{mg\ of\ glucose}{mg\ dry\ matter}$ ($\frac{amount\ of\ polysaccharide}{amount\ of\ polysacharide\ plus\ salts}$) (Anil et al., 2013).

The permeate fraction at the end of the separation consisted 500g of polysaccharide while the retentate fraction consisted 1000g of polysaccharide.

Lyophilization

Dehydration of the polysaccharide slurry is achieved by lyophilization. At first the desalted solution was frozen for 24h at -80°C and after that a Lyophilization phase for two more days. All the process was performed for 3 days in total with the assumption of 100% efficiency, 2.25m^3 of water in total were sublimized.

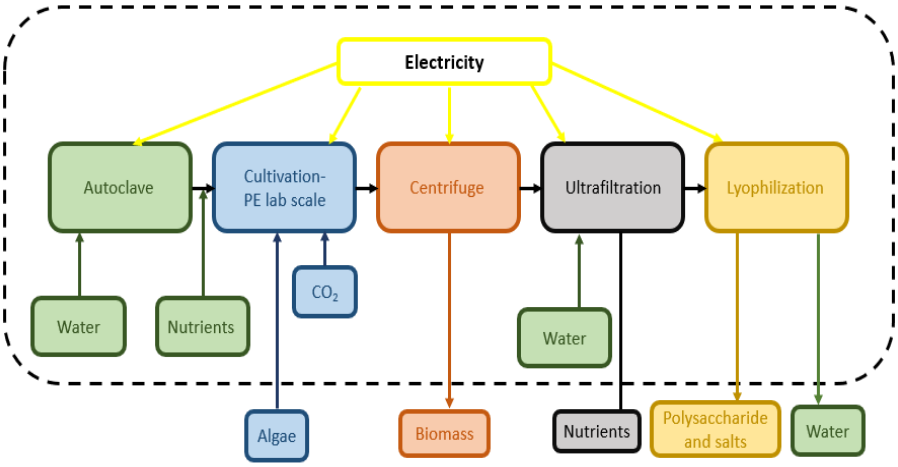

Figure 5: system boundaries of polyethylene sleeves lab scale (scenario A)

2.1.2 Scenario B-polyethylene sleeves pilot scale

Outdoor cultivation of red microalgae in polyethylene sleeves on pilot scale (Cohen et al., 1991). This scenario includes five main stages:

Preparation and sterilization of the medium culture.

Cultivation of the red microalgae

Harvesting the microalgae by centrifuge

Desalting the EPS solution by ultrafiltration

Dry freezing by lyophilization

Preparation and sterilization of the culture medium

The medium contains water and nutrients according to the concentrations mentioned on the introduction.

On this scenario, volume of 11.25m^3 culture medium was required at the beginning for the first run of the reactor, this volume was sterilized by industrial autoclave device for 30min. After the sterilization, the initial inoculum was added 3.75m^3 so that the total volume is 15m^3 The next stage is the cultivation of the microalgae on the bioreactor.

Cultivation of the red microalgae

The total volume of the culture was 15m^3, this volume has been contained in 250 polyethylene sleeves, each 20cm in diameter, 2m high and 60L culture volume (Cohen et al., 1991). The surface area of the bioreactor is 100m^2 containing 5 rows of 50 sleeves each row, with 2m intervals between the rows. The microalgae were grown outdoor with primary concentration of $4 \bullet 10^6$ cells/ml. The cultures were mixed by an air stream containing 3-4% CO_2 pumped into the sleeves at 4-5$L \bullet min^{-1}$ (each sleeve).

The cultures were grown continuously for 40 days in the summer in Elat, Israel, using natural sunlight instead of artificial illumination. In semi continuous mode the production of total polysaccharide is 11.1$g \bullet m^{-2} \bullet day^{-1}$, according to literature (Arad et al., 1988) the soluble polysaccharide constitutes 15% of the total polysaccharide. The production of the biomass is 26.5 $g \bullet m^{-2} \bullet day^{-1}$. To reach 1kg of polysaccharide at the end of the process, at this stage there is a necessity to produce approximately 1.5kg of soluble polysaccharide which takes 9 days of growth.

Harvesting

The soluble polysaccharide and the biomass produced were harvested by industrial centrifuge with an efficiency assumption of 100%. During 40 days of growth the sleeves were harvested 13 times so the harvesting occurred in average once in 3 days. In this experiment 25% of the total volume was harvested and diluted by pure medium, on reaching cell concentration of $20 \bullet 10^6$ cells/ml. To reach 1.5 kg of polysaccharide in total at this stage, the sleeves were harvested 3 times.

Ultrafiltration

The ultrafiltration was performed with automated tangential flow filtration (TFF), membrane with normal molecular weight cut off (NMWCO) of 300KDa. It was performed for 100min using diafiltration technique, desalting 98% of the salt with 15m^3 of water for each batch. The purification that has been reached is 0.57 $\frac{mg\ of\ glucose}{mg\ dry\ matter}$ (Anil et al., 2013).

Lyophilization

Dehydration of the polysaccharide slurry is achieved by industrial lyophilization. At first the desalted solution was frozen for 24h at temperature of -80^{o}C and after that the Lyophilization phase for two more days. The lyophilization was performed for 3 in total with the assumption of 100% efficiency, 11.25m^3 of water in total were sublimized.

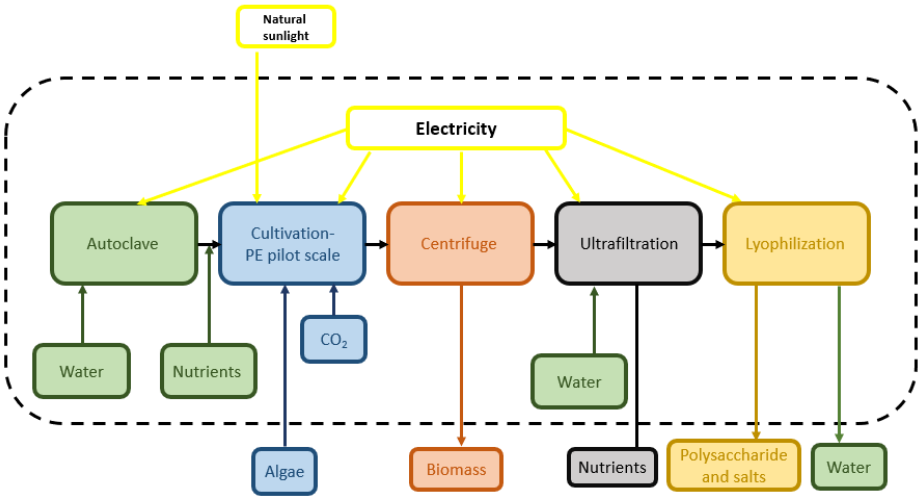

Figure 6: system boundaries of polyethylene pilot scale (scenario B)

2.1.3 Scenario C- open ponds pilot scale

production of 1kg of polysaccharide. Outdoor cultivation of red microalgae in open ponds on pilot scale (Cohen et al., 1991). This scenario includes the same 5 main stages:
Preparation of the culture medium.
Cultivation of the red microalgae
Harvesting the microalgae by centrifuge
Desalting the EPS solution by ultrafiltration
Dry freezing by lyophilization

At this scenario there is no need to sterilize the culture medium.

Preparation of the culture medium

The medium contains water and nutrients according to the concentrations mentioned on the introduction.

On this scenario, volume of 11.25m^3 culture medium was required at the beginning for the first run of the reactor and an initial inoculum of 3.75m^3 was added so that the total volume is 15m^3. The next stage is the cultivation of the microalgae on the bioreactor.

Cultivation of the red microalgae

The total volume of the culture was 15 m^3, this volume has been contained in 1 open pond with 15 cm depth and 100 m^2 surface area. (Cohen et al., 1991).

The microalgae were grown outdoor with primary concentration of $4 \bullet 10^6$ cells/ml and were mixed by paddle wheel. CO_2 was supplied at flow rate of $1.2 L \bullet min^{-1}$. The cultures were grown continuously for 40 days in the summer in Elat, Israel, using natural sunlight during the day and artificial illumination during the night. In semi continuous mode the production of total polysaccharide is $3.6 g \bullet m^{-2} \bullet day^{-1}$, according to literature (Arad et al., 1988) the soluble polysaccharide constitutes 15% of the total polysaccharide. The production of the biomass is 11.4 $g \bullet m^{-2} \bullet day^{-1}$. To reach 1kg of polysaccharide at the end of the process, at this stage there is a necessity to produce approximately 1.5 kg of soluble polysaccharide, which takes 27 days of growth.

Harvesting

The soluble polysaccharide and the biomass produced were harvested by industrial centrifuge with an efficiency assumption of 100%. During 40 days of growth the sleeves were harvested 9 times so the harvesting occurred in average once in 4.5 days. In this experiment 25% of the total volume was harvested and diluted by pure medium, on reaching cell concentration of $20 \bullet 10^6$ cells/ml. To reach 1.5 kg of polysaccharide in total at this stage, the sleeves were harvested 6 times.

Ultrafiltration

The ultrafiltration was performed with automated tangential flow filtration (TFF), membrane with normal molecular weight cut off (NMWCO) of 300KDa. It was performed for 100min using diafiltration technique, desalting 98% of the salt with 15m^3 of water for each batch. The purification that has been reached is 0.57 $\frac{mg\ of\ glucose}{mg\ dry\ matter}$ (Anil et al., 2013).

lyophilization

Dehydration of the polysaccharide slurry is achieved by industrial lyophilization. At first the desalted solution was frozen for 24h at temperature of -80^{o}C and after that the Lyophilization phase for two more days. The lyophilization was performed for 3 in total with the assumption of 100% efficiency, 22.5m^3 of water in total were sublimized.

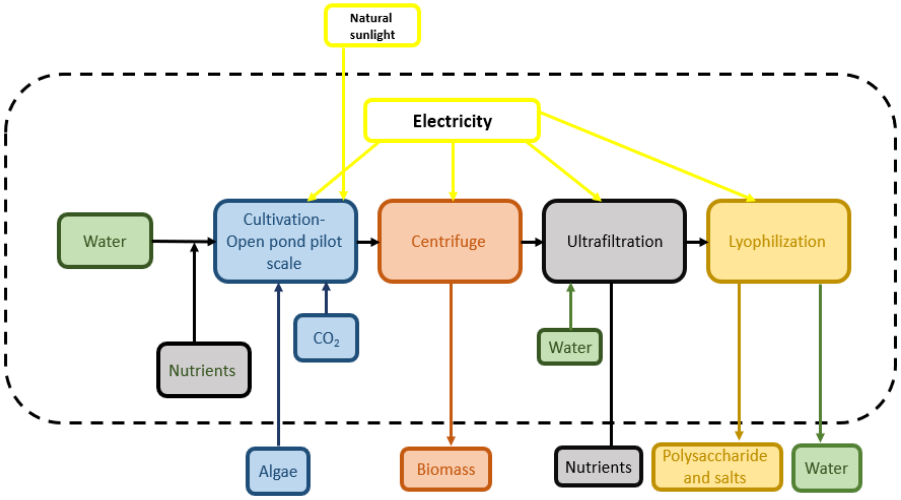

Figure 7: system boundaries of open pond pilot scale (scenario C)

3. RESULTS

3.1 Life cycle inventory

The life cycle inventory data, chemicals and energy consumption consists on a combination between on-site measurements (laboratory in Israel), data from literature and calculations. The data used is regarding the functional unit 1 kg of exo-polysaccharide (EPS) from red microalgae. Table 1 presents the energy consumptions for each stage of the whole process of polysaccharide production from red microalgae of scenario A (polyethylene sleeves lab scale). According to the table 1 microalgae cultivation stage comprises almost all the electricity requirement. The other stages together constitute less than 3% of the total electricity consumption of the process. According to literature (Jez et al., 2017) the algae cultivation stage has the largest electricity requirement. The data about the electricity consumption of the lab scale was collected from a laboratory in Israel (Ester Lalzar-Saban, personal communication). On this case, 60W per 1200 ml medium is required for pumps, temperature monitor and other ancillary equipment. 36W per 1200ml was required for the artificial illumination. The electricity consumption of the illumination alone represents 36% of total power consumption.

Table 1: Energy consumption per 1 kg of EPS for polyethylene sleeves lab scale (scenario A)

Stages on the process	Energy consumption	Units
Autoclave	488	kWh
Cultivation -artificial illumination	18,969	kWh
Cultivation -pumps, temperature monitor etc.	31,616	kWh
Centrifuge	338	kWh
Ultrafiltration	390	kWh
Lyophilization	205	kWh
Total	**52,006**	**kWh**

Table 2 presents the power consumptions of each stage of the whole process of polysaccharide production from red microalgae of scenario B (polyethylene sleeves pilot scale). On this scenario the microalgae cultivation stage has also the largest electricity requirement, the other stages together constitute less than 1% of the total power consumption of the process. The data about the power consumption of the cultivation system was based on the same calculation that has been made for scenario A, because of the lack of information about the system at this scale. This is one of the main limitations of this work. It is suggested that the gathering of detailed energy consumption data is completed in a future work. On this scenario B, the light for the photosynthesis

comes from a natural sunlight instead of artificial illumination, thus a significant amount of energy is saved. Of course, this has an effect on the EPS yield

Table 2: Energy consumption per 1 kg of EPS for polyethylene sleeves pilot scale (scenario B)

Stages on the process	Energy consumption	Units
Autoclave	22.5	kWh
Cultivation	Sun light	
	167,400	kWh
Centrifuge	90	kWh
Ultrafiltration	195	kWh
Lyophilization	430	kWh
Total	**168,137**	**kWh**

Table 3 presents the power consumptions of each stage of the whole process of polysaccharide production from red microalgae of scenario C (open ponds pilot scale). On this scenario C, the microalgae cultivation stage has also the largest electricity requirement, and the other stages together constitute less than 8% of the total power consumption of the process. The data about the power consumption of the paddle wheel in an open pond cultivation was found to be 0.37 kW per 1 m^3 and contribute 33% of the cultivation energy demands (Li et al., 2013). On this scenario C, the irradiation comes from natural sunlight. As mentioned before, this has an effect on the EPS yield. The great advantage of the paddle wheel is the possibility to use a mixing device which request much less electricity for the cultivation than in scenario A and B. As a result, the total energy consumption per unit of mass of EPS is dramatically reduced. Once the energy calculations are described, it is important to highlight additional hypothesis made for the elaboration of the life cycle inventory. One of the assumptions that needed to be made is that almost all of the injected CO_2 (which the cultivation system received) is released back to the atmosphere. Therefore, the contribution of CO_2 to the carbon footprint is zero (only a small fraction of the CO_2 is finally integrated in the biomass and EPS product). In this sense, it was assumed that the production of CO_2 is free of environmental burdens. In future work, it is worthy to explore the possibilities to analyze the origin of the CO_2. Another assumption is that ideal gas in standard conditions was assumed in order to calculate the mass of the carbon dioxide. Finally, the nutrients that are separated from the medium on the ultrafiltration stage are not recycled back to the medium. The destination of the

biomass produced is not clear neither in the literature nor in the selected references so it will be assumed that is just an organic waste that can be managed without problems.

Table 3: Energy consumption per 1 kg of EPS for open ponds pilot scale (scenario C)

Stages on the process	Power consumption	Units
Cultivation	Sun light	kWh
	10,782	kWh
Centrifuge	180	kWh
Ultrafiltration	390	kWh
Lyophilization	304	kWh
Total	**11,656**	**kWh**

As a result of the mass and energy balances performed for the three scenarios described, a life cycle inventory for each scenario is described next. The inventory, stating the nutrients and salts, water, CO_2 and electricity consumption for the process of EPS production on polyethylene sleeves on Lab scale (Scenario A) is shown in table 4. The CO_2 excess may explain the reason behind the greatest concentration of EPS 0.66 $g \cdot L^{-1}$ at the cultivation stage in this scenario in comparison to the other scenarios, as the amount of CO_2 improves the probability that the algae would fix it. The injected CO_2 is incorporated both to the cell mass and of course in a lesser extension to the production of EPS. The unused CO_2 is returned to the atmosphere as mentioned before. The injection of CO_2 guarantees a proper excess which promotes the growth of the cells and the production of the extracellular product.

Table 4: Energy and materials consumption per 1 kg of EPS for polyethylene sleeves lab scale (scenario A)

Input	Value	Units
Total electricity consumption	52,006	kWh
Water	11.3	m^3
Nutrients	95	Kg
CO_2	18.63	t

Table 5: Energy and materials consumption per 1 kg of EPS for polyethylene sleeves pilot scale (scenario B)

Input	Value	Units
Total electricity consumption	168,137	kWh
Water	59.7	m^3
Nutrients	627	Kg
CO_2	0.79	t

The inventory, nutrients and salts, water, CO_2 and electricity consumption (as shown in table 5) for the process of polysaccharide production regarding to the functional unit on polyethylene sleeves pilot scale (Scenario B). As can be seen that the power consumption of the system is very high.

Table 6: Energy and materials consumption per 1 kg of EPS for open ponds pilot scale (scenario C)

Input	Value	Units
Total electricity consumption	11,656	kWh
Water	11.9	m^3
Nutrients	1254	Kg
CO_2	91.6	Kg

The inventory, nutrients and salts, water, CO_2 and electricity consumption (as shown in table 5) for the process of polysaccharide production regarding to the functional unit on polyethylene sleeves pilot scale. The carbon dioxide injection per volume of culture medium is the lowest on this scenario so is the electricity consumption.

3.2 Life cycle impact assessment

The quantities of nutrients, water and electricity consumptions for the production process were estimated to determine the environmental impacts regarding to the emissions of greenhouses gases. The emission factors that had been taken of the electricity consumption sources are 0.737 and 0.05 CO_2-eq/kWh for the Israeli mix grid and the photovoltaic solar energy respectively.
Concerning nutrients impact on the global warming, it was calculated assuming that the nutrients are not recycled back to the medium. Their impact calculated with respect to the contribution of each nutrient to CO_2 emission.

Table 7: Carbon footprint per 1 kg of EPS for polyethylene sleeves lab scale (scenario A) by using two sources of electricity consumption, Israeli mix grid and photovoltaic

Input	Carbon footprint using the Israel grid mix	Carbon footprint using PV	Units
Power consumption	38,328	2,600	Kg CO_2-eq.
Water	4	4	Kg CO_2-eq.
Nutrients	24	24	Kg CO_2-eq.
Total	**38,356**	**2,648**	**Kg CO_2-eq.**

Results in table 7 represent the contribution of each parameter of the inputs to carbon footprint.

As it was expected, the greatest contribution was the electricity consumption of the process, due to this critical impact two sources of electricity consumption have been chosen for the three scenarios; PV and mix grid of Israel.

Table 8: Carbon footprint per 1 kg of EPS for polyethylene sleeves pilot scale (scenario B) by using two sources of electricity consumption, Israeli mix grid and photovoltaic

Input	Carbon footprint using the Israel grid mix	Carbon footprint using PV	Units
Power consumption	123,917	8,407	Kg CO_2-eq.
Water	19	19	Kg CO_2-eq.
Nutrients	154	154	Kg CO_2-eq.
Total	**124,090**	**8,580**	**Kg CO_2-eq.**

Results in table 8 represent the contribution of each parameter of the inputs to the greenhouse gases emission with two sources of power consumption.

The greatest contribution was the electricity consumption of the process.

On this scenario due to the large power consumption the rest of the inputs barely effect on the carbon footprint.

Table 9: Carbon footprint per 1 kg of EPS for open ponds pilot scale (scenario C) by using two sources of electricity consumption, Israel grid mix and photovoltaic

Input	Carbon footprint using the grid mix Israel	Carbon footprint using PV	Units
Power consumption	8,590	583	kg CO_2-eq.
Water	37	37	kg CO_2-eq.
Nutrients	308	308	kg CO_2-eq.
Total	**8,935**	**928**	**kg CO_2-eq.**

Results in table 9 represent the contribution of each parameter of the inputs to the greenhouse gases emission with two sources of power consumption. Although the water consumption in this scenario C is the greatest among those discussed, it barely affects the environment regarding to the global warming impact. The carbon footprint is the lowest on this scenario when the PV is used as source of power consumption.

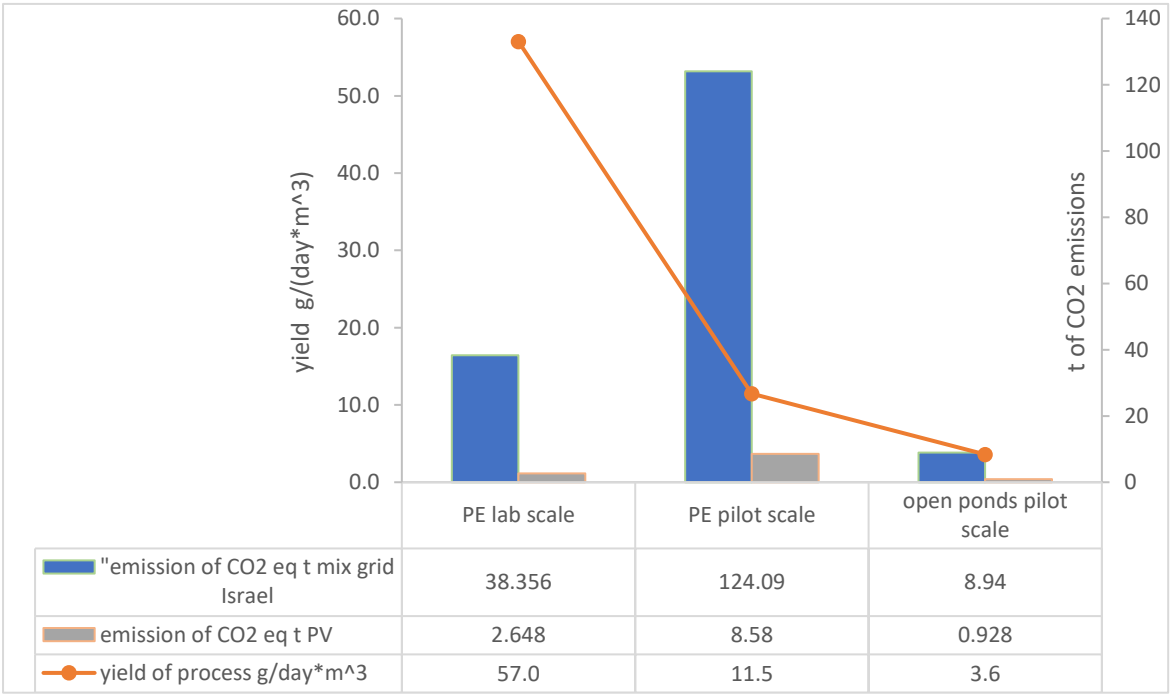

Figure 8: scenario and source of electricity consumption effect on greenhouse gases emission

Results on figure 8 show the effect of the source of electricity consumption on the carbon footprint regarding the present scenarios. Clearly, for each scenario, the PV modules contribute less than the Israeli mix grid to the global warming, which is expected as almost no fossil fuels are used. It also

can be said that the cultivation system determines the environmental impact regarding to global warming due to the fact that the critic parameter is the power consumption. The cultivation system is by far the largest consumer of electricity; thus, it can have stated that the energy consumption in this stage determines the carbon footprint profile of the EPS. Regarding to the yield of the process, the polyethylene sleeves lab scale has the greatest yield, due to the controlled conditions, the continuous artificial sunlight and the excess of CO_2. The open pond has the lowest carbon footprint because of the lowest electricity consumption even if it has the lowest yield relatively to the other scenarios A and B using the PE sleeves. The polyethylene sleeves pilot scale system cultivation has the highest carbon footprint due to the high-energy consumption in the cultivation stage. As it was previously mentioned, it is recommend analyzing with real data the actual consumption of electricity as it was scaled up from the data in scenario A.

4. DISCUSSION

Polysaccharide from red microalgae is a bio-substance that allows wide range of functional properties that can be utilized on multiple industries instead of synthetic substances that destined to the same purposes. Production of polysaccharide from red microalgae is an alternative source that needed to be found because of the intensive harvesting and ecological damage due to the increasing demands of colloids from seaweeds. No studies of life cycle assessment regarding to the production of polysaccharide from red microalgae are known in literature. Therefore, there is an interest in this kind of high value products.

This study of life cycle assessment was made in order to satisfy the main goal to assess the carbon footprint associated with the production of 1 kg sulfated cell wall polysaccharide from red microalgae, so the emission of greenhouse gases was estimated respect to the presented scenarios at three different scales. On the study the raw materials, the CO_2 that is required for the cultivation stage, the water and the electricity consumption (inputs) for all the stages were taken into account for the estimation of greenhouse gases emissions. Within the process, there is a separation between the polysaccharide from the biomass but no allocation procedure was required because it is an organic waste. The three presented scenarios were:

Scenario A - polyethylene sleeves in lab scale

This scenario includes five main stages to produce 1 kg of soluble polysaccharide, the cultivation system is closed system comprised of transparent polyethylene sleeves in lab scale. The equipment and devices that have been used are in laboratory scale. The data regarding to this scenario is based on laboratory measurements in Israel, data from literature and calculations.

In order to produce 1 kg of polysaccharide an assumption of linear connection between the volume of culture medium and the polysaccharide production was made. The same assumptions were made for CO_2, power consumptions etc.

Scenario B- polyethylene sleeves in pilot scale

This scenario includes five main stages to produce 1 kg of soluble polysaccharide, the cultivation system is closed system comprised of transparent polyethylene sleeves in pilot scale. The data regarding to this scenario is based on literature data and additional calculations.

Scenario C- open ponds in pilot scale

The scenario includes five main stages to produce 1 kg of soluble polysaccharide, the cultivation system is an open system in pilot scale. The data regarding to this scenario is based on literature data and additional calculations.

Note that for the different scenarios there are different cultivation conditions e.g. rate of CO_2 per volume of medium, artificial/natural illumination, the cultivation system itself etc., so the growth of microalgae and the yield of polysaccharide production are different. Results in figure 8 showed that the yields are 57.0, 11.5 and 3.6 $g \cdot m^{-3} days^{-1}$ for scenarios A, B and C respectively. The yield of polyethylene sleeves in lab scale is the greatest of the three scenarios as expected because of the controlled and more suitable conditions. The open ponds have the lowest yield as expected "algal cultivation in sleeves is superior to that in open ponds with respect to growth and production" (Arad, et al., 1991). According to the results, the production of polysaccharide and the growth of algae it seems that polyethylene sleeves in lab scale and pilot scale are superior than open ponds. However. The possibility to use low-intensive mixing devices such as the paddle wheel helps at reducing the carbon footprint.

The results in tables 7-9 showed that almost all GHG emissions were associated to the production of electricity requirements. The most crucial parameter is the power consumption, therefore, changing the source of the electricity consumption was performed. The electricity production is based on the Israel grid mix and photovoltaic solar energy (PV). The photovoltaic was chosen because of its modularity and the current decreasing costs. According to the results in figure 8, the carbon footprint is the highest in the polyethylene sleeves at pilot scale relatively to open ponds and polyethylene sleeves lab scale. Using the Israel grid mix, the carbon footprint per kg of EPS are 124 t $CO_{2\,eq}$ for the PS pilot scale and 8.94 t and 38.3 t for OP pilot scale and PS lab scale respectively. Using PV as source of power consumption, the carbon footprint per kg of EPS are 8.6 t $CO_{2\,eq}$ for the PS pilot scale and 0.93 t and 2.65 t for OP pilot scale and PS lab scale respectively.

Due to the fact that there is a yield loss of the process with the increasing extensive magnitude, the volume of the microalgae cultivation, there is a need for more electricity for the polyethylene sleeves pilot scale to increase the yield. The open pond seems to be superior to the PE systems due to the lowest GHG emission since it requires less energy for the operation.

According to literature (López et al., 2017), to produce 30.7 kg of biomass (the amount that could be produced by the same conditions and resources) in open ponds, 9,827 kWh are required for

cultivation, thus to produce 1 kg of polysaccharide 10,789 kWh are required. According to this, it can be stated that the energy consumption used in this work is acceptable. According to (Scherer et al., 2017), the carbon footprint of the production of 10 g biomass in closed cultivation system is 58.3 kg $CO_{2\ eq}$, therefore, the carbon footprint of producing 24.7 kg of biomass (the amount that could be produced by the same conditions and resources) would be 144 t with leaner connection hypothesis. The carbon footprint of polyethylene sleeves pilot scale is 124 t. Because of the different hypothesis and the lack of additional references, it can be stated that the carbon footprint for the EPS obtained in this work for the different scenario is acceptable.

Experiments and studies that have been made during the years indicate that there are problems in large scale cultivation of algae in open ponds. The low productivity and high contamination relatively to closed and controlled systems as the polyethylene sleeves are some of these problems. Although productivity of 46 $g \cdot m^{-2} \cdot day^{-1}$ polysaccharide has been achieved in open ponds on small scale for short times, the same quantities could not be achieved on a large scale for long periods of time. The major reason why there is a problem with open ponds on large scale is because the relatively high contamination that causes collapse of cultures (Cohen et al., 1989)

5. CONCLUSIONS

This works presents the carbon footprint of the production of exo-polysaccharide by red microalgae at different scales. As far as the author is concerned, it is the first time this environmental analysis is applied for this specific product. In the regard of the expected large environmental impact associated to the unit of mass of product, a carbon footprint study is envisaged as a necessary step in the product commercialization. Because of the findings of this work, it can be stated the next set of conclusions:

Cultivation in open ponds is superior than in polyethylene sleeves regarding to carbon footprint due to the lower energy consumption in the cultivation stage. The cultivation of algae on large scale open ponds for long periods of time can be subject to other issues such as external contamination (which is not the subject of this work).

Even if the yield at large scale is lower than in lab or pilot scale, the low energy consumption by the paddle wheel in the open ponds is worthy in energy terms.

Being the electricity consumption the key issue in the environmental profile, the use of PV is strongly recommended to decrease the values from 124 t CO_2-eq per kg of EPS in the PE pilot scale (using the Israel grid mix) to 3.6 kg CO_2-eq per kg of EPS in the open ponds cultivation (using PV solar energy).

Future work regard to the need to complete in-situ inventories for high quality inventory.

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