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Evaluation of preliminary plant design for *Chlorella vulgaris* microalgae production focused on cosmetics purposes

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Evaluation of preliminary plant design for *Chlorella vulgaris* microalgae production focused on cosmetics purposes

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Abstract. Although there are currently different large-scale microalgae production systems, its performance is subject to high production cost problems. That is why the main goal of this research is analyzing the feasibility of obtaining the *Chlorella vulgaris* microalgae from a proposed preliminary industrial plant design, considering its use in the cosmeceutical industry as skin care. Parameters used in the SuperPro Designer simulations were programmed based on experimental data reported from bibliography. For biomass and cell extract production, a Bold Basal enriched medium at 1% with soy flour was used. It is found that mass flow significantly affects production costs since a product value of 8.58 USD/ml was estimated operating the plant with 20 kg/h of mass flow. Interestingly 84 % unit reduction cost was improved by operating the proposed plant at 140 kg/h mass flow since 1.34 USD/ml of cosmetic product was determined. These findings show the potentiality of *Chlorella vulgaris* to be positioned in the world market. The latter, by considering its dependence on a successfully large scale plant. The above, in order to identify potential improvements focused on maximizing productivity and profits at the lowest operating cost.

1. Introduction

Algae are a simple-structure aquatic group of organisms producing oxygen from photosynthesis process and convert light energy into chemical energy for synthesis of organic molecules. These microorganisms are of great importance for different industries such as agriculture, food, environmental, pharmaceutical and cosmetics. Pharmaceutical and cosmetic sector has been forced to reinvent itself due to multiple factors based on economy changes. The latter did a chance to cosmeceuticals. Products considered cosmeceuticals contain biologically active ingredients for aesthetic purposes. Simultaneously, these antioxidant metabolites are characterized for a high biochemical capacity on the skin. This property causes the cosmeceutical to act in a more active and effective way on skin tissue. Convergence of cosmetics and aesthetic medicine sector has been the reason for the rise of these products in recent years, to create a new sector focused on image. That is why, both beauty centers and aesthetic clinics are more and more expanded in global markets. The use of algae in cosmetic products has been in great demand due to its high content of trace elements, mineral salts, vitamins and amino acids that serve to maintain the skin good appearance, since they are directly assimilable by skin cells. The latter, considering the world market value of products based on natural cosmetics, which reached a value of almost 34.50 trillion dollars in 2018 and a rate of 54.50 trillion is expected for 2027 [1]. Regarding Chlorella, sales have been growing and the global Chlorella market has reached more than $ 138 million in 2016 and is expected to reach $ 164 million in 2021 [3]. Currently, there are more than 70 industrial companies that produce around 2000 tons of Chlorella products every year [1-2].

The largest individual Chlorella producer is located in Taiwan, which produces 400 tons/year, followed by a German company with 130-150 tons/year [1]. These data are proof of the growing importance of the natural cosmetics market, especially from the use of Chlorella for cosmeceutical purposes. The above, taking into account its ability to produce different bioactive molecules with antioxidant capacity such as carotenoids, chlorophyll, tocopherols, ubiquinone and proteins. [3-4]. Thus, Chlorella microalgae is one of the most interesting species that promote collagen synthesis in the skin, tissue regeneration through joint repair such as sports injuries and cardiovascular protection, among others [5]. It is also known that it can decrease the wear and tear of arterial collagen and prevent diseases such as atherosclerosis. However, the high costs involved in obtaining the micro-alga *Chlorella vulgaris* have been mainly addressed through the use of economic and profitable substrates [6-8]. It is for these reasons that there is a need for a successfully plant design allowing the growth maximization of *Chlorella vulgaris* micro-alga for cosmetic purposes. The SuperPro Designer computational tool is one of the most complete and recognized process design software packages, being a very versatile simulator that can satisfy the needs of engineers in a wide variety of industries, such as Biotechnology, Pharmaceutical, Chemical, Food, Mining, Wastewater Treatment, Environmental Control, etc. That is why the purpose of this research is to evaluate the productivity of the *Chlorella vulgaris* microalgae from a computational approach, performing a simulation using SuperPro Designer software to identify the technical requirements of the process from data obtained from bibliography.

2. Methodology

For the large-scale simulation of *Chlorella vulgaris* the following generalized production phases are proposed (Figure 1) considering the upstream processing (unit preparation operations): (a) Culture media preparation, (b) sterilization, (c) inoculation train and (d) cell growth. Similarly, for the downstream stages (bio-separation and formulation) until obtaining a product based on an emulsion for topical use, the following unit operations are proposed: (e) centrifugation, (f) cell disruption and (g) packaging.

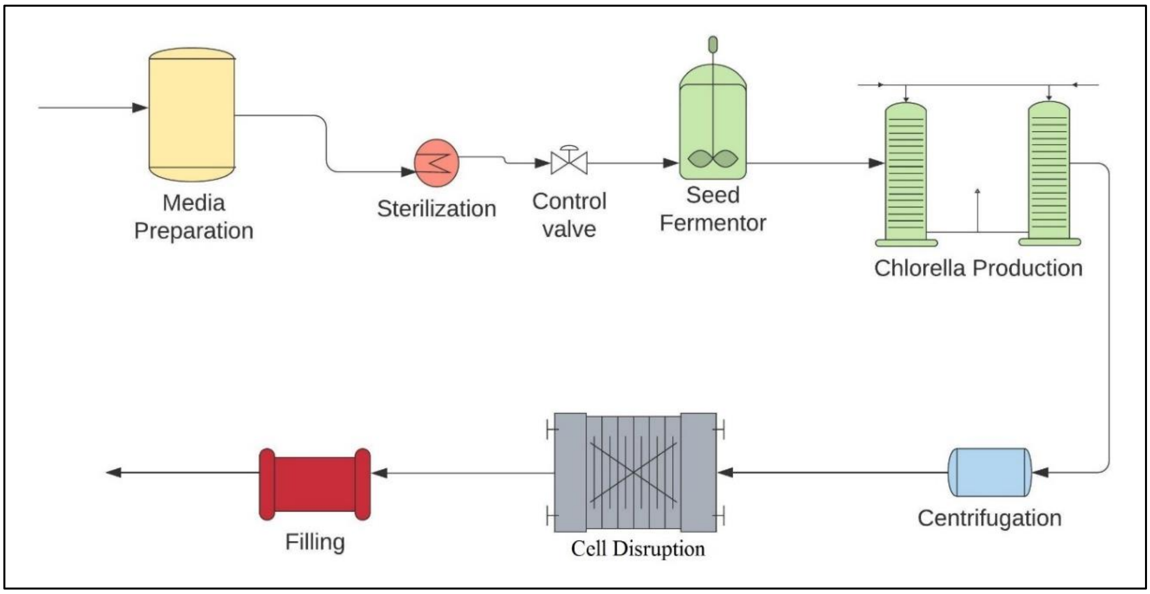

Figure 1. Generalized unit operations diagram for large scale *Chlorella vulgaris* production.

2.1. Culture medium preparation and sterilization

The culture medium used for the simulations was reported from the literature [9], and is defined by the following composition enriched with soy flour at 1%: 0.57 g/L of $(NH_4)_2SO_4$, 0.057 g/L of $CaCl_2$, 0.17 g/L of $MgSO_4$, 0.40 g/L of KH_2PO_4, 0.057 g/L of NaCl, 2.4 g/L peptone and 1.4 g/L of glucose.

In this research, a continuous operating mode heat sterilization is performed for removing any presence of unwanted microorganisms.

2.2. Biomass cell growth

For *Chlorella vulgaris* inoculum growth a scale up process was carried out using seed fermenters. Subsequently, a production fermentation is proposed for the large scale culturing with a temperature ranging at 35 ºC. The operating conditions were adjusted based on the bibliographic references.

2.3. Biomass separation and downstream processing

The out stream leaving production fermenter tank is treated using a centrifuge for biomass removal from supernatant. This procedure was set up using a centrifuge [3]. Then, the product is pumped to a bead milling for cell disruption processing, in order to break the cells and obtain the intracellular metabolites. Therefore, micro-filtration unit operation is carried out to obtain a cleaner and more concentrated product. Also, a recirculation stream is proposed for product recovering.

3. Results and Discussions

The main goal of this research is analyzing the feasibility of obtaining the *Chlorella vulgaris* microalgae from a proposed preliminary industrial plant design, considering its use in the cosmeceutical industry as skin care. Mass flow effect on unit operating is considered at starting point for the profitability analysis. The latter, for further comparison related to similar products in the current market. Operating conditions as well as culture media and biomass yields were set up according to bibliographic references. Figure 2 shows the specific process diagram set up in SuperPro Designer Software for large scale *Chlorella* production proposed for a cosmetic industry by-aesthetic products.

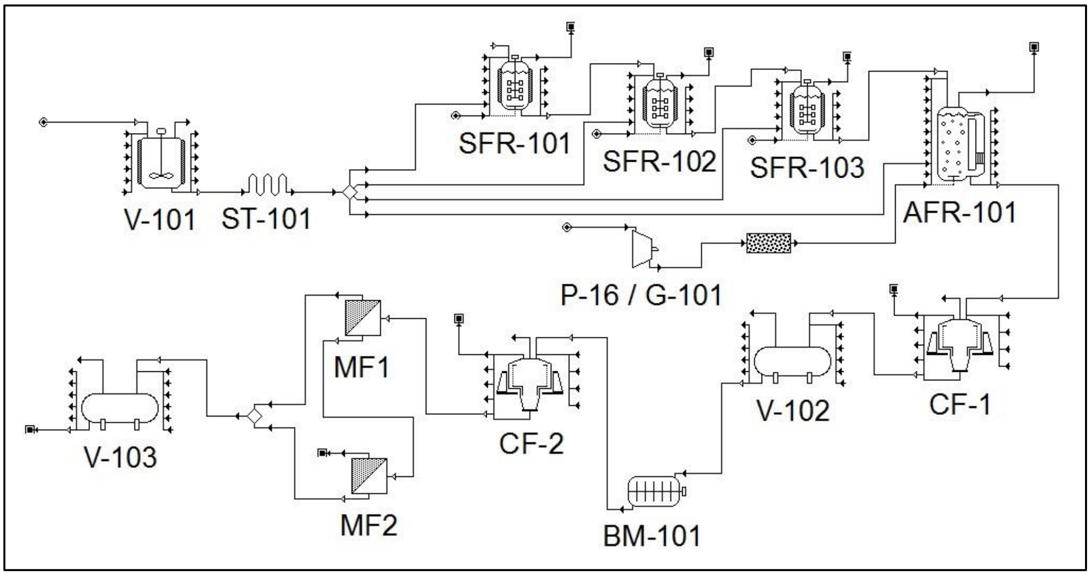

Figure 2. Industrial plant Scheme proposed for *Chlorella vulgaris* production.

As shown in Figure 2, different equipment is set up for large scale Chlorella production: V-101, V-102 and V-103 are mixing/storage tanks for different purposes, ST-101 is an installed heat sterilization system for removing contaminants before cell growth, SFR-101, SFR-102 and SFR-103 account for seed fermenters, AFR-101 is the air lift bioreactor, CF-1 and CF-2 are centrifuges, BM-101 is a bead milling for cell disruption and MF1 and MF2 are microfiltration modules.

Tables 1-3 show the streams resulted from different culture medium [9] mass flow (20-140 kg/h) set up at proposed industrial plant. Results of biomass produced under mass flow feed are presented regarding outlet stream of the industrial-scale air-lift AFR -101 type fermenter (see Table 1).

Table 1. Out Stream calculations at airlift bioreactor for large scale cosmetic production (20-140 kg/h).

Component	S-118 (Cell Growth)			
Mass Flow	20 kg/h	60 kg/h	100 kg/h	140 kg/h
Biomass	0.04	0.13	0.22	0.31
Glucose	0.01	0.02	0.04	0.06
Fat	0.04	0.12	0.20	0.28
Minerals	0.01	0.03	0.05	0.07
Peptone	0.00	0.01	0.01	0.01
Proteins	0.07	0.21	0.35	0.49
Water	19.81	59.43	99.06	138.68
TOTAL (kg/h)	19.99	59.96	99.93	139.91
TOTAL (L/h)	20.17	60.52	100.87	141.21
Component	S-122 (Centrifuge I)			
Mass Flow	20 kg/h	60 kg/h	100 kg/h	140 kg/h
Biomass	0.04	0.13	0.22	0.31
Water	0.25	0.75	1.26	1.76
TOTAL (kg/h)	0.30	0.89	1.49	2.08

According to estimated results and considering the soy flour as a limiting substrate, it is possible to obtain yields in the range between 0.20-0.22 g biomass/g substrate using the SuperPro Designer software. These calculations are based on previous experiments referenced from bibliography as input parameters [6].

Once the biomass containing the active ingredients from *Chlorella vulgaris* has been obtained, it must be separated from the outlet stream of the airlift-type bioreactor. Therefore, at this stage of the process a centrifuge CF-1 is proposed that allows solid recovery. Then, out stream from centrifugation is pumped to storage tank V-102 and treated to obtain the cosmetic product. Table 2 shows the results.

However, biomass recovered must be processed to release its intracellular content which act as antioxidants and collagen skin stimulator. These intracellular metabolites represent extremely attractive for biotechnological and cosmetic companies [10, 11].

At this stage, molecules such as carbohydrates, lipids, and intracellular proteins [5] are released by installing a homogenizer BM-101 that performs a cell disruption process (see Table 2). However, this stream must be re-treated in a centrifuge and micro-filtration equipment in order to remove the cell debris or biomass residues resulting from the cell disruption stage.

For this reason, a second centrifugation stage CF-2 and a coupled micro-filtration system (MF-101-MF-102) are proposed in this research to treat the centrifugal output stream (S-130). The results are observed in Table 3.

Table 2. Out Streams at centrifugation I and cell disruption (20-140 kg/h).

Component	S-122 (Centrifuge I)			
Mass Flow	20 kg/h	60 kg/h	100 kg/h	140 kg/h
Biomass	0.04	0.13	0.22	0.31
Water	0.25	0.75	1.26	1.76
TOTAL (kg/h)	0.30	0.89	1.49	2.08
TOTAL (L/h)	0.30	0.89	1.48	2.08
Component	S-128 (Cell Disruption)			

Mass Flow	20 kg/h	60 kg/h	100 kg/h	140 kg/h
Biomass	0.00	0.00	0.01	0.01
Carbohydrates	0.01	0.03	0.04	0.06
Fiber	0.01	0.03	0.04	0.06
Lipids	0.00	0.01	0.02	0.03
Proteins intra.	0.02	0.06	0.11	0.15
Water	0.25	0.75	1.26	1.76
TOTAL (kg/h)	0.30	0.89	1.49	2.08
TOTAL (L/h)	0.30	0.89	1.49	2.08

Table 3. Out Streams at centrifugation II, Micro-filtration and Storage (20-140 kg/h).

Component	S-130 (Centrifuge II)			
Mass Flow	20 kg/h	60 kg/h	100 kg/h	140 kg/h
Carbohydrates	0.01	0.02	0.03	0.05
Lipids	0.00	0.01	0.02	0.02
Proteins intra.	0.02	0.05	0.09	0.12
Water	0.20	0.60	1.00	1.40
TOTAL (kg/h)	0.23	0.68	1.14	1.60
Component	S-136 (UltraFiltration)			
Mass Flow	20 kg/h	60 kg/h	100 kg/h	140 kg/h
Carbohydrates	0.01	0.02	0.03	0.04
Lipids	0.00	0.01	0.02	0.02
Proteins intra.	0.02	0.05	0.08	0.12
Water	0.19	0.58	0.97	1.35
TOTAL (kg/h)	0.22	0.66	1.10	1.53
Component	S-125 (Product)			
Mass Flow	20 kg/h	60 kg/h	100 kg/h	140 kg/h
Carbohydrates	0.01	0.02	0.03	0.04
Lipids	0.00	0.01	0.02	0.02
Proteins intra.	0.02	0.05	0.08	0.12
Water	0.19	0.58	0.97	1.35
TOTAL (kg/h)	0.22	0.66	1.10	1.53

Protein intracellular content recovered from *Chlorella vulgaris* cells are estimated at levels of 0.02 kg/h during plant operation at 20 kg/h (see streams S-136 and S-125). In contrast, levels of intracellular protein recovered for cosmetic product increase with mass flow until reaching values close to 0.12 kg/h. The latter, as explained below, is a promising value for profitability of the product in the market. Operation volume required for preliminary design of a production plant (see Figure 3-a) is one of the most important design parameters to consider, since the latter, defines the production capacity. In the case of cosmetic purposes, the production capacity is measured as Productivity (*Qp*). Results of these parameters are shown in Figure 3 (b).

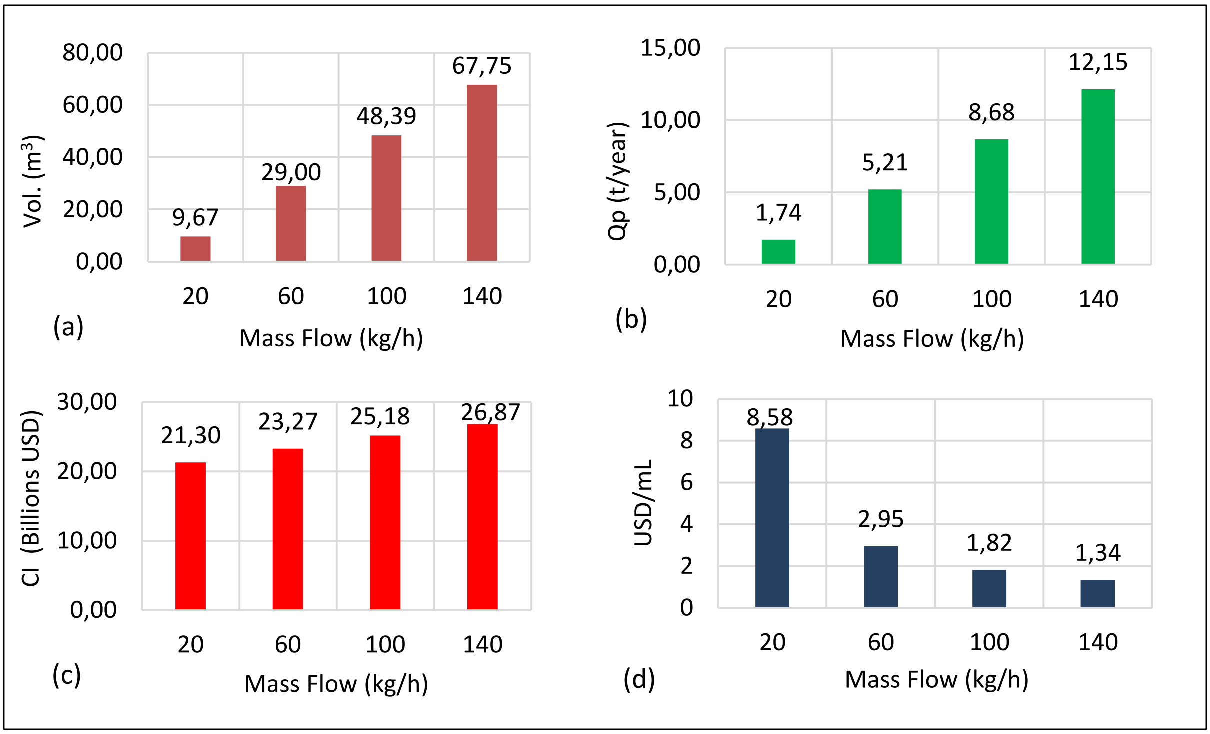

Figure 3. Parameters determination for large scale *Chlorella* production: (a) Bioreactor volume required (m^3), (b) *Qp* productivity reached, (c) Capital investment (CI) and (d) Unit operating cost.

According to the proposed plant fed different culture medium mass flow (20-140 kg/h), annual productivity values were estimated in the range between 1.74-12.15 tons of microalgae extract for cosmetic purposes. However, to achieve the studied productivities according to calculations simulated by SuperPro Designer, a capital investment, comprised between 21.30 - 26.87 billion dollars (see Figure 3-c) is suggested. It is worth mentioning that this investment resource refers to the purchase of equipment, adjustments for its operation, installation and construction of required infrastructure for cosmetic purposes.

Currently, it is possible to get cosmetics extracts obtained from *Chlorella vulgaris* on markets from Amazon and Susanne Kaufmann web sites, among others, with values ranging close to 4.34 USD/mL. According to the results obtained in Figure 3 (d), the most profitable results could be established by operating the proposed plant with a mass feed flow between 100-140 kg/h. The above, supported by the results obtained from the unit operating cost, with values between 1.34-1.82 USD/mL. It is worth mentioning that to achieve these objectives, the operating volume must also be modified between 48-70 m^3.

Operating costs distribution result from the determination of costs associated with culture media, electricity, steam, personnel, equipment usage time, laboratory analysis, treatment of wastewater, among others [12]. Results calculated based on each parameter are shown in Figure 4.

As explained before, results suggest significant effect of mass flow on unit operating cost. Cost fraction associated with personnel is reduced from 65% (20 kg/h) to a level of 60% (140 kg / h) regarding all sceneries studied.

However, costs associated with equipment use (facilities), are increased regarding mass flow effect. The latter considering, equipment maintenance, insurance policies, local taxes, and depreciation costs. According to the results shown in this research, a large scale *Chlorella* technology for cosmetic purpose would be a profitable process at operating mass flow of 100-140 kg/h. The latter would assure a competitive price in the market.

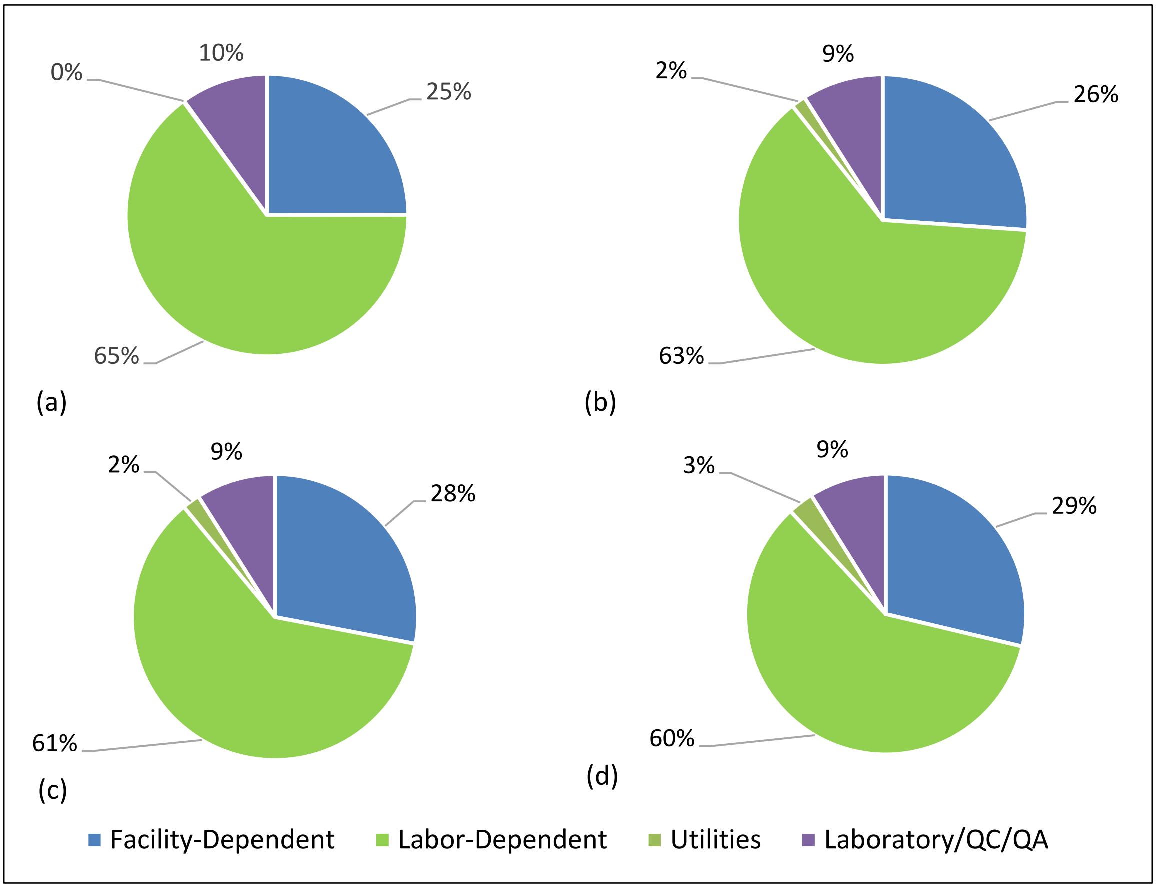

Figure 4. Annual Operating cost estimations for *Chlorella* large scale production based on mass flow effect: (a) 20 kg/h, (b) 60 kg/h, (c) 100 kg/h and (d) 140 kg/h.

4. Conclusions

Through the research carried out, it was possible to design a biomass and metabolite production plant for cosmetic sector. The findings found here demonstrate the importance of predicting a large-scale bioprocess focused on improving productivity. Culture medium mas flow has a significant effect on cost reduction. A production volume of 48-70 m^3 is required to reach similar viable values to those reported in the literature.

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