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Faculty of Mechanical Engineering
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Bachelor thesis

Laboratory pearl mill

Erasyl Kadyr

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Annotation list

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The bachelor thesis will solve the following items:

Preparation of a review scoped on the problematics of microalgae's cell wall disintegration with the emphasis on the application of pear mill (i.e. bead mill), including the review and comparison of efficiencies and energy requirements of individual techniques. The main task is to design a laboratory bead mill, which is planned to be used for disintegration of microalgae's cell wall. Also, the thesis includes a performance of the necessary design, process and strength calculations, and the design documentation for the unit.

Keywords: Bead mill, algae, cell wall disruption.

I declare, that this bachelor thesis was fully elaborated myself and independently, except the guidance of my supervisor. I listed all the sources and references of the literature.

In Prague_____   Signature_____

Appreciation

I would like to thank all the teachers, who gave me knowledge which I have by this time. After all this time studying in our department with you, I realized how unwise I am. You do truly righteous job.

Also, I want to express deep gratitude to my supervisor Lukáš Krátký for his support and patience. For motivation and all the time he unreservedly spent with me. It was my pleasure to gain this experience with you. Thank you!

Content

1. Introduction

Humanity nowadays strongly depends on energy, and not only on supply, but on consequences of usage. Production, manufacturing, transportation are only a few examples of this dependency. Consequences of irrational use of energy in its part might become much more difficult challenge to overcome. Global warming, polar ice melting, rice of ocean's level and other succession of negative outcomes occur everywhere in globe. Hopefully, in the 21st century progress does not stand still. Amount of knowledge based on many researches have formed lots of different ways to work on. Alternative energy sources seem to be perspective solutions.

What sources do you imagine when think about alternative energy? Solar and wind energy, geothermal and marine hydrokinetic energy, maybe biofuels. To get closer to the topic of my thesis I would like to talk about bioenergy. Bioenergy is renewable energy made available from organic materials derived from biological sources. This set of sources is called biomass. Biomass is any organic material which has stored sunlight in the form of chemical energy. As a fuel it includes wood, wood waste, straw, manure, sugarcane, algae and many other bio products from a variety of agricultural processes. Algae fuel or algal biofuel is an alternative to liquid fossil fuels that uses algae as its source of energy-rich components, practically lipids (table 2). Like fossil fuels and other corn and sugarcane based biofuels, algae fuel releases CO_2 when burnt, but unlike fossil fuel, biofuels release only recently removed from atmosphere via photosynthesis as the plant grew. One of algal fuels' attractive characteristics is that algae can be grown using lands and water unsuitable for agriculture which means absence of the choice whether to grow food or fuel sources. Possibility of production in saline and wastewater means minimal impact on fresh water resources. Algae fuel is biodegradable and relatively harmless to the environment if spilled. It has also got a high flash point. Algae cost more per unit mass than other biofuels due to high capital and operating costs, but yield above ten times more fuel per unit area. [1] It yields 4-6 times more often than sugarcane and CO_2 absorption is 3-6 times higher. Table 1 shows yields of microalgae in comparison to other traditional biofuel sources. The United States Department of Energy estimates that if algae fuel replaced all of the petroleum fuel in the USA, it would require only 39000 square kilometers, which is 0.4% of the country's area and 1/7 of the area of corn harvested in the US in 2000. This is very impressive, considering the fact that the US consumes almost 25% of global petroleum production.

Chevron Corporation, one of the largest multinational energy corporations, has started researches in possibility of use algae to produce fuel, particularly jet fuel. Big companies such as Honeywell UOP have recently started a project in production of algal based jet fuel for military purposes. Tokyo Gas Co. the largest gas provider in Japan intends to build a power plant. Methane released form disintegrated algae will be used to generate electricity. For set of Japanese prefectures, including the area of the capital city,

coast pollution by algae remains a serious problem. They stink and deteriorate the landscape. The plan above is considered to solve the pollution issue by an economically advantageous way. [2]

1.1 Algae classification

Marine algae are classified into macroalgae and microalgae. Macroalgae include red algae, brown algae and green algae, while microalgae include chlorella and spirulina, etc. The worldwide annual marine algae production is approximately 14 million tons and is expected to increase more than 22 million tons in 2020. [3]

Seaweeds or macroalgae belong to the lower plants meaning that they do not have roots, stems and leaves. Instead they are composed of a thallus (leaf-like) and sometimes a stem and a foot. Some species have gas-filled structures to provide buoyancy. They are subdivided into three groups, the red, green and brown macroalgae. [4, p. 5]

Micro-algae are microscopic photosynthetic organisms that are found in both marine and freshwater environments. Their photosynthetic mechanism is similar to land based plants, but due to a simple cellular structure, and submerged in an aqueous environment where they have efficient access to water, CO_2 and other nutrients, they are generally more efficient in converting solar energy into biomass. [5, p. 238] The dominating species of microalgae exploitation for bioenergy generation includes *Isochrysis*, *Chaetoceros*, *Chlorella*, *Arthrospira* (*Spirulina*) and *Dunaliella.* [4, p. 11]

Crop	Oil yield (gallons/acre)
Corn	18
Soybean	48
Canola	127
Jatropha	202
Coconut	287
Palm oil	636
Microalgae	6283 - 14641

Table 1. Comparison of oil content and oil yield for different sources of biofuel [6]

Microalgae	Oil content (% dry cell weight)
Botryococcus braunii	25-75
Chlorella sp.	28-32
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	55-77
Tetraselmis suecia	15-23

Table 2. Oil content of microalgae [6]

1.2 Production and agronomic cultivation.

Most microalgae are strictly photosynthetic — that is, they need a light and carbon dioxide as energy and carbon sources. This culture mode is usually called photoautotrophic. Some algae species, however, are capable of growing in darkness and using organic carbons such as glucose or acetate as energy and carbon sources. This culture mode is termed heterotrophic. Due to high capital and operational costs, heterotrophic algal culture is hard to justify for biodiesel production. In order to minimize costs, algal biofuel production usually relies on photoautotrophic culture that uses sunlight as a free source of light. [7]

The most important parameters regulating algal growth are nutrient quantity and quality, light, pH, turbulence, salinity and temperature. Optimal range of these parameters you can see below in table 3.

Parameters	Range	Optima
Temperature (C^0)	16-27	18-24
Salinity (g/l)	12-40	20-24
Light intensity (lux)	1000-10000 (depends on volume and density)	2500-5000
Photoperiod (light/dark, in hours)		min: 16/8 max: 24/0
pH	7-9	8.2-8.7

Table 3. A generalized set of conditions for culturing micro-algae [8]

Also, the various factors may be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for another. [9, p. 227]

Phototrophic microalgae require light, carbon dioxide, water, and inorganic salts to grow. The culture temperature should be between 15 and 30°C for optimal growth. Since concentrations of cells in phytoplankton cultures are generally higher than those found in nature the growth medium must

contribute the inorganic elements that help make up the algal cell, such as nitrogen, phosphorus, iron, and sometimes silicon. [6]

Silicate is specifically used for the growth of diatoms which utilize this compound for production of an external shell. Micronutrients consist of various trace metals and the vitamins thiamin (B_1), cyanocobalamin (B_{12}) and sometimes biotin. Two enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne medium (Table 4.1) and the Guillard's $F/_2$ medium (Table 4.2) [8]

Constituents	Quantities
Solution A (at 1 ml per liter of culture)	
Ferric chloride ($FeCl_3$)	0.8 g[a]
Manganous chloride ($MnCl_2$, $4H_2O$)	0.4 g
Boric acid (H_3BO_3)	33.6 g
EDTA[b], di-sodium salt	45.0 g
Sodium di-hydrogen orthophosphate (NaH_2PO_4, $2H_2O$)	20.0 g
Sodium nitrate ($NaNO_3$)	100.0 g
Solution B	1.0 ml
Make up to 1 litre with fresh water[c]	Heat to dissolve
Solution B	
Zinc chloride ($ZnCl_2$)	2.1 g
Cobaltous chloride ($CoCl_2$,$6 H_2O$)	2.0 g
Ammonium molybdate (($NH_4)_6Mo_7O_{24}$, $4H_2O$)	0.9 g
Cupric sulphate ($CuSO_4$, $5H_2O$)	2.0 g
Concentrated HCl	10.0 ml
Make up to 100 ml fresh water[c]	Heat to dissolve
Solution C (at 0.1 ml per liter of culture)	
Vitamin B_1	0.2 g
Solution E	25.0 ml
Make up to 200 ml with fresh water[c]	
Solution D (for culture of diatoms-used in addition to solutions A and C, at 2 ml per liter of culture)	
Sodium metasilicate (Na_2SiO_3, $5H_2O$)	40.0 g
Make up to 1 litre with fresh water[c]	Shake to dissolve
Solution E	
Vitamin B_{12}	0.1 g
Make up to 250 ml with fresh water[c]	
Solution F (for culture of *Chroomonas salina* - used in addition to solutions A and C, at 1 ml per liter of culture)	
Sodium nitrate ($NaNO_3$)	200.0 g
Make up to 1 litre with fresh water[c]	

Table 4.1 Composition and preparation of Walne medium [8]

Nutrients	Final concentration $(mg.l^{-1}$ seawater)[a]	Stock solution preparations
$NaNO_3$	75	**Nitrate/Phosphate Solution** Working Stock: add 75 g $NaNO_3$ + 5 g NaH_2PO_4 to 1 liter distilled water (DW)
$NaH_2PO_4.H_2O$	5	
$Na_2SiO_3.9H_2O$	30	**Silicate Solution** Working Stock: add 30 g Na_2SiO_3 to 1 liter DW
$Na_2C_{10}H_{14}O_8N_2.H_2O$ (Na_2EDTA)	4.36	**Trace Metal/EDTA Solution** Primary stocks: make 5 separate
$CoCl_2.6H_2O$	0.01	1-liter stocks of $(g.l^{-1}$ DW) 10.0 g $CoCl_2$, 9.8 g
$CuSO_4.5H_2O$	0.01	$CuSO_4$, 180 g $MnCl_2$, 6.3 g Na_2MoO_4, 22.0 g $ZnSO_4$
$FeCl_3.6H_2O$	3.15	
$MnCl_2.4H_2O$	0.18	Working stock: add 1 ml of each primary stock solution + 4.35 g $Na_2C_{10}H_{14}O_8N_2$ + 3.15 g $FeCl_3$ to 1 liter DW
$Na_2MoO_4.2H_2O$	0.006	
$ZnSO_4.7H_2O$	0.022	
Thiamin HCl	0.1	**Vitamin Solution** Primary stock: add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B_{12} to 1 liter DW
Biotin	0.0005	
B_{12}	0.0005	Working stock: add 5 ml primary stock to 1 liter DW

Table 4.2 Composition and preparation of Guillard's F/2 medium [8]

Light

As with all plants, micro-algae photosynthesize, *i.e.* they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard intensity, spectral quality and photoperiod need to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture (*e.g.* 1,000 lux is suitable for Erlenmeyer flasks, 5,000-10,000 is required for larger volumes). Light may be natural or supplied by fluorescent tubes. Too high light intensity (*e.g.* direct sun light, small container close to artificial light) may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided. Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis. The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develop normally under constant illumination. [8]

pH value

The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. The latter is accomplished by aerating the culture (see below). In the case of high-density algal culture, the addition of carbon dioxide allows to correct for increased pH, which may reach limiting values of up to pH 9 during algal growth. [8]

Aeration/mixing

Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (*e.g.* in outdoor cultures) and to improve gas exchange between the culture medium and the air. The latter is of primary importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. For very dense cultures, the CO_2 originating from the air (containing 0.03% CO_2) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply (*e.g.* at a rate of 1% of the volume of air). CO_2 addition furthermore buffers the water against pH changes as a result of the CO_2/HCO_3^- balance. Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test tubes, erlenmeyers), aerating (bags, tanks), or using paddle wheels and jetpumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing. **[8]**

Temperature

The optimal temperature for phytoplankton cultures is generally between 20 and 24°C, although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of micro-algae tolerate temperatures between 16 and 27°C. Temperatures lower than 16°C will slow down growth, whereas those higher than 35°C are lethal for a number of species. If necessary, algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air - conditioning units. [8]

Salinity

Marine phytoplankton are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tap water. Salinities of 20-24 g.l^{-1} have been found to be optimal. [8]

Sources of contamination and water treatment

Contamination with bacteria, protozoa or another species of algae is a serious problem for axenic cultures of micro-algae. The most common sources of contamination include the culture medium (sea water and nutrients), the air (from the air supply as well as the environment), the culture vessel, and the starter culture. Seawater used for algal culture should be free of organisms that may compete with the unicellular algae, such as other species of phytoplankton, phytophagous zooplankton, or bacteria. Sterilization of the seawater by either physical (filtration, autoclaving, pasteurization, UV irradiation) or chemical methods (chlorination, acidification, ozonization) is therefore required. Autoclaving (15 to 45 min. at 120°C and 20 psi(1.37 bar), depending on the volume) or pasteurization (80°C for 1-2 h) is mostly applied for sterilizing the culture medium in test tubes, erlenmeyers, and carboys. Volumes greater than 20 l are generally filtered at 1 μm and treated with acid (*e.g.* hydrochloric acid at pH 3, neutralization after 24 h with sodium carbonate) or chlorine (*e.g.* 1-2 mg.l^{-1}, incubation for 24 h without aeration, followed by aeration for 2-3 h to remove residual chlorine, addition of sodium thiosulfate to neutralize chlorine may be necessary if aeration fails to strip the chlorine). Water treatment is not required when using underground salt water obtained through bore holes. This water is generally free of living organisms and may contain sufficient mineral salts to support algal culture without further enrichment. In some cases well water contains high levels of ammonia and ferrous salts, the latter precipitating after oxidation in air. A common source of contamination is the condensation in the airlines which harbor ciliates. For this reason, airlines should be kept dry and both the air and the carbon dioxide should be filtered through an in-line filter of 0.3 or 0.5 μm before entering the culture. For larger volumes of air, filter units can be constructed using cotton and activated charcoal (figure 1). The preparation of the small culture vessels is a vital step in the upscaling of the algal cultures:

· wash with detergent

· rinse in hot water

· clean with 30% muriatic acid

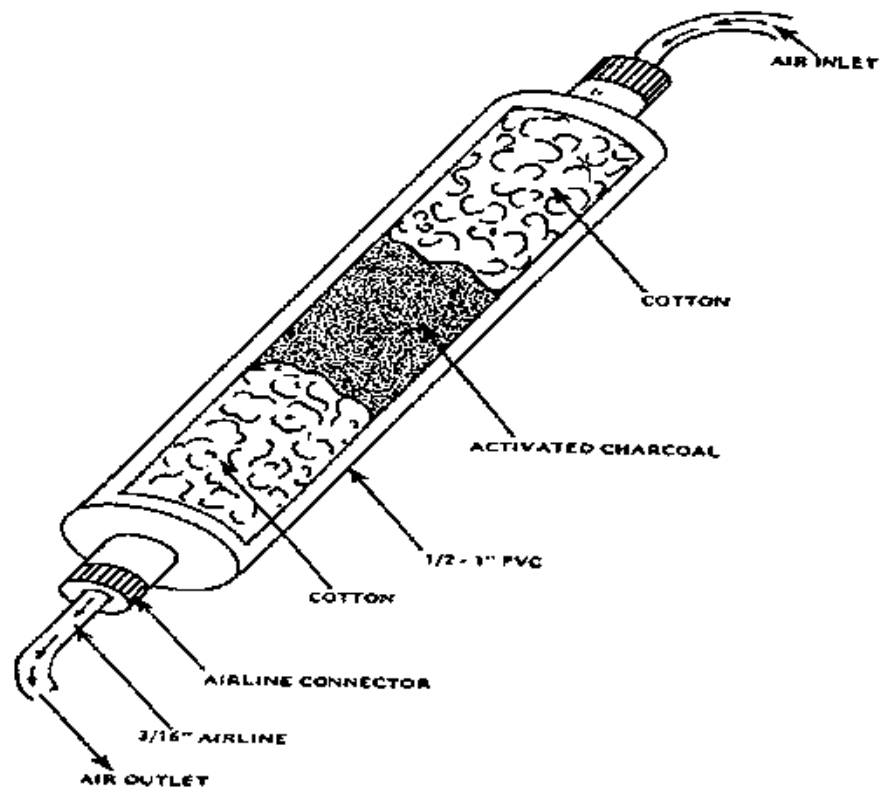· rinse again with hot water

· dry before use. [8]

Fig1 Filter unit, based on activated charcoal and cotton [8]

Alternatively, tubes, flasks and carboys can be sterilized by autoclaving and disposable culture vessels such as polyethylene bags can be used. [8]

For large-scale production of microalgae, algal cells are continuously mixed to prevent the algal biomass from settling, and nutrients are provided during daylight hours when the algae are reproducing. However, up to one-quarter of algal biomass produced during the day can be lost through respiration during the night.

A variety of photoautotrophic-based microalgal culture systems are available. For example, the algae can be grown in suspension or attached on solid surface. Each system has its own advantages and disadvantages. Currently, the suspension-based open ponds and enclosed photobioreactors are commonly used for algal biofuel production. In general, an open pond is simply a series of raceways outside, while a photobioreactor is a sophisticated reactor design which can be placed indoors in a greenhouse, or outdoors. [6]

Open ponds: In this system, the shallow pond is usually about 30 centimeters deep; algae are cultured under conditions identical to their natural environment. The pond is designed in a raceway configuration, in which a paddlewheel provides circulation and mixing of the algal cells and nutrients. The raceways are typically made from poured concrete, or they are simply dug into the earth and lined with plastic to prevent the ground from soaking up the liquid. Baffles in the channel guide the flow around bends in order to minimize space. Figure 2 illustrates schematic of the open pod system for algae culture. The system is often operated in a continuous mode — that is, the fresh feed containing nutrients including nitrogen phosphorus and inorganic salts is added in front of the paddle wheel. Algal broth is harvested behind the paddle wheel after it has circulated through the loop. [6]
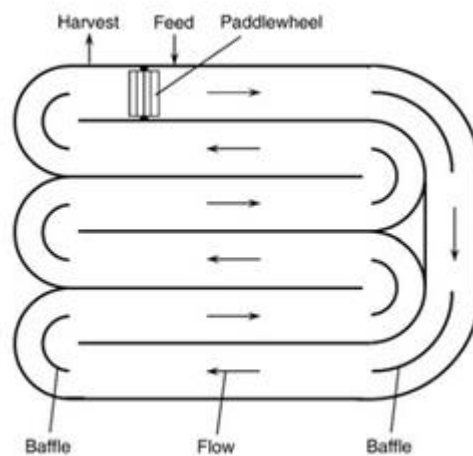

Figure 2.Schematic open pond system for algae culture [7]

Enclosed photobioreactors: Enclosed photobioreactors have been employed to overcome the contamination and evaporation problems encountered in open ponds. These systems are made of transparent materials and generally placed outdoors for illumination by natural light. The cultivation vessels have a large surface area-to-volume ratio.

The most widely used photobioreactor is a tubular design (figure 3), which has a number of clear transparent tubes, usually aligned with the sun rays. The tubes are generally less than 10 centimeters in diameter to maximize sunlight penetration. The medium broth is circulated through a pump to the tubes, where it is exposed to light for photosynthesis, and then back to a reservoir. The algal biomass is prevented from settling by maintaining a highly turbulent flow within the reactor, using either a mechanical pump or an airlift pump. A portion of the algae is usually harvested after the solar collection tubes. In this way, continuous algal culture is possible. In some photobioreactors, the tubes are coiled spirals to form what is known as a helical tubular photobioreactor, but these systems sometimes require artificial illumination, which adds to the production cost. Therefore, this technology is only used for high-value products, not biodiesel feedstock. [10, p. 54]
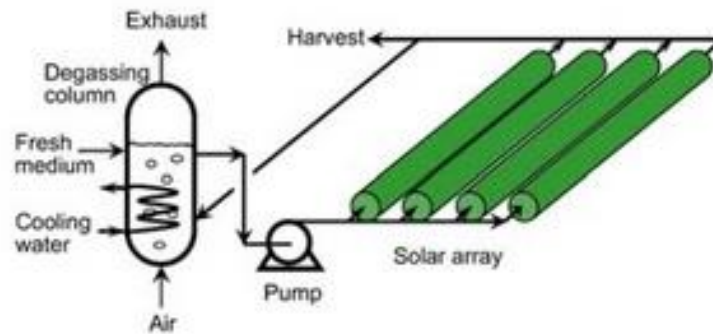
Figure 3. Schematic tubular photobioreactor [7]

The photosynthesis process generates oxygen. In an open-raceway system, this is not a problem as the oxygen is simply returned to the atmosphere. However, in the closed photobioreactor, the oxygen levels will build up until they inhibit and poison the algae. The culture must periodically be returned to a degassing zone, an area where the algal broth is bubbled with air to remove the excess oxygen. Also, the algae use carbon dioxide, which can cause carbon starvation and an increase in pH. Therefore, carbon dioxide must be fed into the system in order to successfully cultivate the microalgae on a large scale. Photobioreactors may require cooling during daylight hours, and the temperature must be regulated at night hours as well. This may be done through heat exchangers, located either in the tubes themselves or in the degassing column. [10, p. 55]

The advantages of the enclosed photobioreactors is that they can overcome the problems of contamination and evaporation encountered in open ponds. The biomass productivity of photobioreactors can be 13 times greater than that of a traditional raceway pond, on average. Harvesting of biomass from photobioreactors is less expensive than that from a raceway pond, since the typical algal biomass is about 30 times as concentrated as the biomass found in raceways. However, enclosed photobioreactors also have some disadvantages: the reactors are more expensive and difficult to scale up. Moreover, light limitation cannot be entirely overcome since light penetration is inversely proportional to the cell concentration. Attachment of cells to the tube walls may also prevent light penetration. Although enclosed systems can enhance the biomass concentration, the growth of microalgae is still suboptimal due to variations in temperature and light intensity. [10, p. 55]

Harvesting: After growing in open ponds or photobioreactors, the microalgae biomass needs to be harvested for further processing. The commonly used harvest method is through gravity settlement, or centrifuge. The oil from the biomass will be removed through solvent extraction and further processed into biodiesel. [6]

Advantages of Algae Oil as a FUEL FEEDSTOCK: One gallon of algae oil can be made into one gallon of biodiesel fuel, compared to a 42 gallon barrel (160 liters) of crude petroleum can only produce 6

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gallons (23 liters) of diesel fuel. This gives algae roughly a 5 to 1 advantage over the use of crude oil when calculating the raw feedstock needed to achieve a particular level of fuel production. Current feedstock production rates for "standing crops" such as Soy Beans, Camelina, Rape Seed, and Jetropha are in the 200 to 400 gallon per acre per year range. Palm Oil is a little better with 400 to 700 gallons per acre per year. These production rates fall far short of the production rates per acre of Algae which is currently delivering up to 60000 gallons (227 cubic meters) of oil per acre per year from a variety of types and configurations of production systems. [11]

One of the good advantages of biodiesel compared to other biofuel feedstock is that it can be used in modern engines without modifications of those. As biodiesel production continues to grow, it can be distributed by the same infrastructure, replacing petroleum diesel without critical adjustments in our lifestyle. Not only does this eliminate the chicken-egg problem, making biodiesel far way feasible than hydrogen but also excludes the huge-cost changes in worldwide fuel distribution infrastructure.

1.3 Economics of algal biofuel production:

The production cost of algal oil depends on many factors, such as yield of biomass from the culture system, oil content, scale of production systems, and cost of recovering oil from algal biomass. Currently, algal-oil production is still far more expensive than petroleum diesel fuels. For example, Chisti (2007) estimated the production cost of algae oil from a photo bioreactor with an annual production capacity of 10,000 tons per year. Assuming the oil content of the algae to be approximately 30 percent, the author determined a production cost of $2.80 per liter of algal oil. This estimation did not include costs of converting algal oil to biodiesel, distribution and marketing costs for biodiesel, and taxes. At the same time, the average worldwide petroleum-diesel price is less than one US dollar per liter. Whether algal oil can be an economic source for biofuel in the future is still highly dependent on the petroleum oil price. Chisti (2007) used the following equation to estimate the cost of algal oil where it can be a competitive substitute for petroleum diesel:

$$C_{algal\ oil} = 25.9 \times 10^{-3}\ C_{petroleum}$$

where: $C_{algal\ oil}$ is the price of microalgae oil in dollars per gallon and $C_{petroleum}$ is the price of crude oil in dollars per barrel.

This equation assumes that algal oil has roughly 80 percent of the caloric energy value of crude petroleum. For example, with petroleum priced at $50 per barrel, algal oil should cost no more than $1.3 per gallon in order to be competitive with petroleum diesel. [7]

With respect to photosynthetic efficiency, there appears to be a general consensus that up to 9% of incident solar energy can be converted to biomass, this equates to an efficiency of 27% of PAR (photosynthetically active radiation, about 45% of total light). This value corresponds to a productivity of somewhat over 300 t ha-1 y-1 of conventional land plants (Benemann and Oswald 1996). This productivity has never been achieved in practice.

Current and reproducible yields for algae are in the 20 – 50 t ha-1 y-1 range, as demonstrated by the Aquatic Biomass Program. Higher values have been observed under closely controlled conditions in short-duration experiments (for example Lee and Low 1991). However, these conditions (turbulent mixing, low cell density, optimal temperature and medium light intensity below levels causing saturation or inhibition) cannot be transferred to commercially viable large-scale systems. [4, p. 20]

For micro-algae the productivity of raceway ponds and photo bioreactors is limited by a range of interacting issues. While it may be possible to tackle individual issues, it has not yet been possible to effectively combine the solutions. This may be because the solutions are simply not additive, are mutually exclusive, or because of escalating associated costs. For example, several possible target areas to improve productivity in large-scale installations have been proposed as follows:

1. *Culture depth or optical cross section*: thinner tubes or shallower ponds have been suggested to improve growth rates since algal cultures progressively absorb light and cause shading for algae at lower depths/inside tubes.

2. *Mixing*: greater turbulence would theoretically bring all cells into brighter light

3. *Nutrient content and supply*: nitrogen and CO2 can be optimized

4. *Cultivation procedure*: batch, (semi)-continuous or multistage processes.

5. *Photosynthetic system*: reduced antenna size leading to higher quantum yields.

However, all of these solutions that have been suggested suffer from major drawbacks. For example, increased population densities improve light utilization but require thorough mixing to avoid mutual shading: all cells should be exposed regularly to bright light. This can be accomplished by reducing the light path (thin tubes, shallow ponds), but this reduces the effective volume per surface area, and increases the cost per product unit. Thorough mixing may in principle be used to expose all cells to bright light regularly. However, to benefit from the flashing light phenomenon (which operates on the microsecond timescale), the required turbulent flow would lead to an energy input that exceeds energy output by far. [4, p. 21]

2. Microalgae cell wall disruption techniques:

Microalgae provides much higher oil yields than traditional crops and oil-rich waste, that is a good point to use it as an alternative to petrochemical products. But for algae based biodiesel to compete with petroleum diesel one must use the most efficient techniques in all the aspects beginning from algae's growth up to the distribution to the market Figure 4. One of the critical part is oil extraction. In order to maximize the final product. We have got to avoid inefficient, high energy demanded operations. For this reason, I would like to review general techniques for algae cell wall disruption.
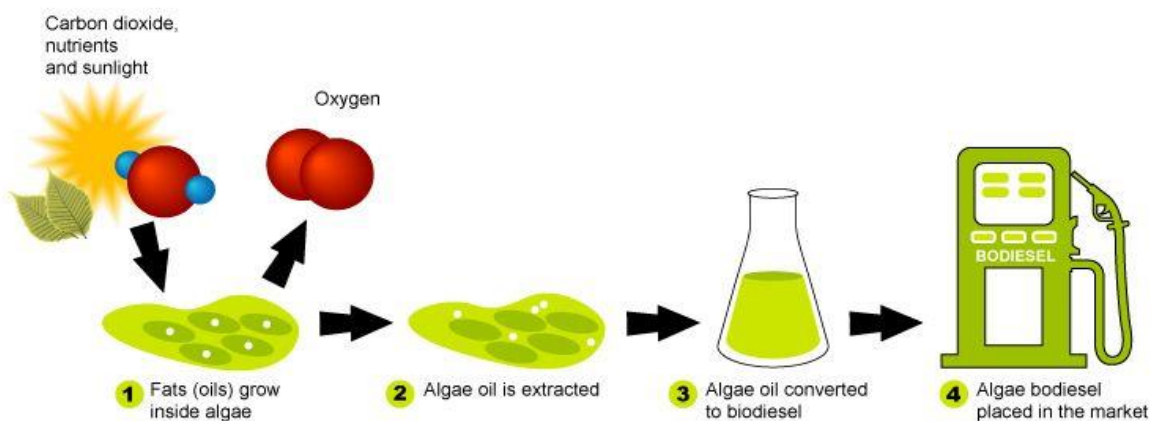
Figure 4. Scheme of fundamental processes involved into algae based biodiesel production [12]

The downstream process of microalgae biorefineries involves a number of various steps in which cell wall disruption takes essential part of it.

Disruption of the algal cells follows after the drying process to release the products of interest. Microbial cells including algal cells can adapt to environmental changes and could withstand resistance to disruption which is a salient ability of microorganisms for adaptation. To efficiently extract materials from the inside of cells, some particular form of cell disruption is generally required. There are several ways to disrupt microalgal cell wall. Depending on the algae wall characteristics and the product nature, we can choose the proper one according to our purpose. The key criterion here is to maximize the value of the materials obtained from the processes, which means that rapid and precise disruption should be used. In an industrial setting, an appropriate cell disruption technology is selected based on the durability of the cell walls to be disrupted, the size of the process stream, the risk of sub-cellular destruction of important products, the costs of the process and the safety concerns. [13]

A variety of disruption methods are currently designed for cell disruption of cellular walls and membranes in order to release the content of the cell. All of these methods have both benefits and drawbacks. In general way the techniques can be divided into two groups: mechanical and non-mechanical (Figure 5).
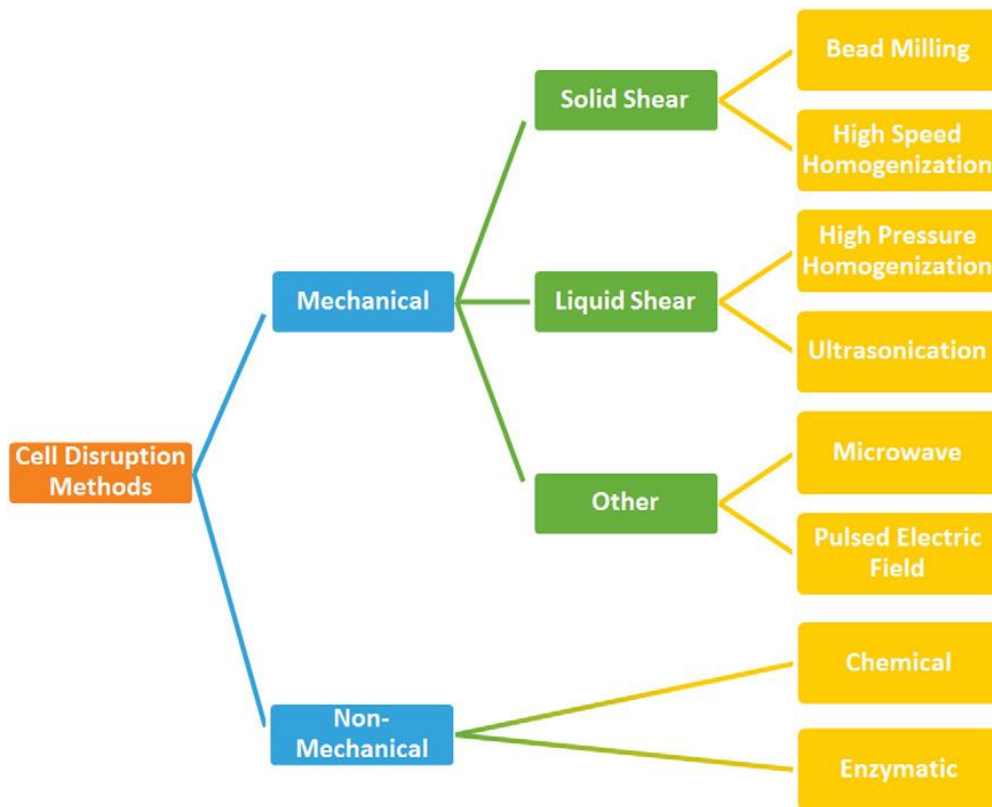
Figure5. Process classification for algal cell wall disintegration [13]

2.1 Mechanical methods

Mechanical disruption methods includes Bead Milling, High pressure and High speed Homogenization, Ultrasonication, Pulsed Electric Field. Let me refer to excellent description of the techniques, named above by research review paper. Table 5 shows an overview of the parameters affecting the disruption yield for the different mechanical and non-mechanical cell disruption methods:

Disruption method	Mechanism of cell disruption	Process parameters
Bead milling	Mechanical compaction and shear stress	Agitation disk design, speed Bead filling, size, material Dry weight Feed rate Growth phase and conditions Microalgae type Time Cooling
High pressure homogenization	Cavitation and shear stress	Cycle number Dry weight Flow rate Growth phase and conditions Homogenizator design Microalgae type

Disruption method	Mechanism of cell disruption	Process parameters
		Pressure
High speed homogenization	Cavitation and shear stress	Blade design, speed Dry weight Growth phase and conditions Microalgae type Time
Ultrasonication	Cavitation and free radical formation	Cycle number and time Dry weight Growth phase and conditions Microalgae type Power of ultrasound
Microwave treatment	Temperature increase, molecular energy increase	Agitation Dry weight Growth phase and conditions Microalgae type Power of microwave Time
Pulsed electric field treatment	Proliferation due to electricity	Conductivity (electrolyte concentration) Current Dry weight Growth phase and conditions Microalgae type Oscillation Time

Disruption method	Mechanism of cell disruption	Process parameters
Enzymatic lysis	Enzyme substrate interaction	Agitation Dry weight Enzyme concentration Enzyme type Growth phase and conditions Microalgae type Oxygen level Type and amount of buffer Temperature Pressure Time
Chemical treatment	Chemical substrate interaction	Agitation Chemical concentration Chemical type Dry weight Growth phase and conditions Microalgae type Temperature Time

Table 5. Process parameters of the cell disruption methods. [13]

2.1.1 Bead milling (also known as pearl milling, ball milling)

High disruption efficiency in single-pass operations, high throughput, high biomass loading, good temperature control, commercially available equipment, easy scale up procedures, and low labor intensity are the primary factors that make bead milling an interesting cell disruption method with high potential for industrial implementation. The most common design for this system is shown in figure 6.
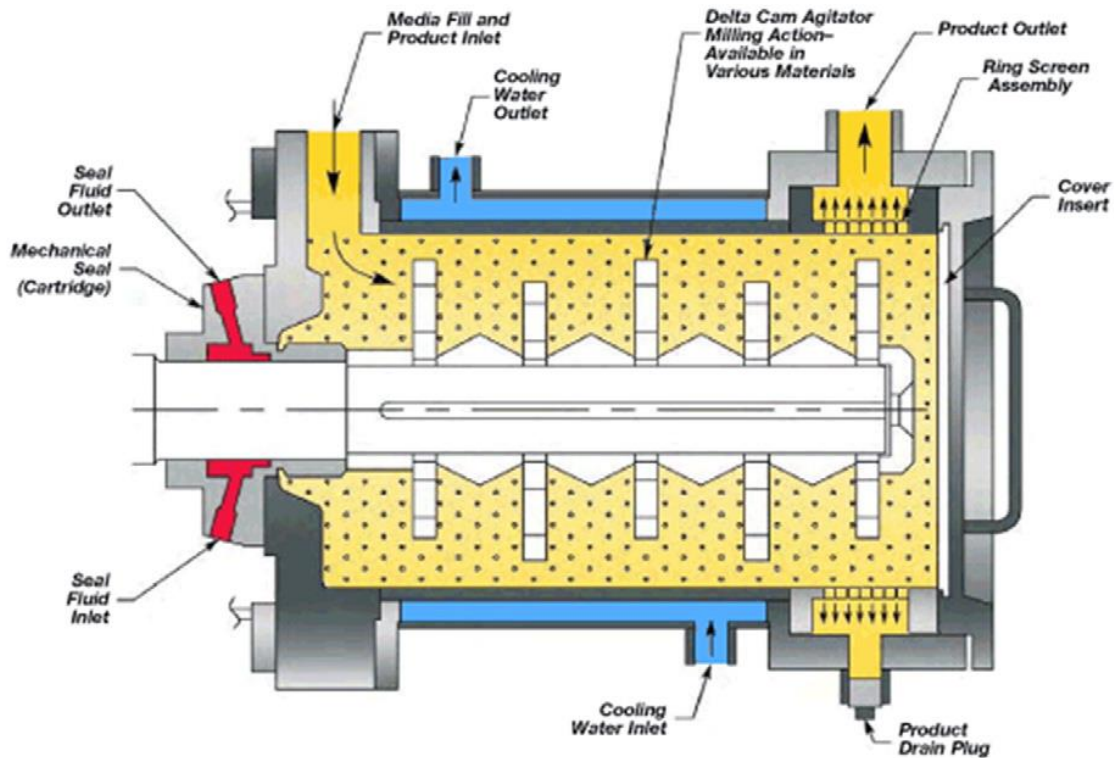

Figure 6. Bead mill (pearl mill) [13]

The shaft may carry agitators of varied design (concentric or eccentric disks or rings) that export kinetic energy to small steel, glass or ceramic beads in the chamber resulting in multiple collisions. It is hypothesized that the suspended cells are disrupted in the bead collision zones by compaction or shear forces with energy transfer from the beads to the cells. [13]

Based on the results of the case studies, it is concluded that increasing the bead diameter has a positive effect when the beads are smaller than 0.5 mm and has a negative effect above 0.5 mm. Additionally, high density beads (e.g., zirconium) are more effective in media with high viscosity while low density beads (e.g., glass) are preferred in low viscous media. Increasing the treatment time, agitator peripheral speed (tip speed 5–10 m·s^{-1}), number of cycles and bead filling up to 85% of grinding chamber volume have a positive effect on the disruption process. Increasing dry cell weight (DCW; 0.5–8% w/w) and biomass flow rate (kg DCW/ h) negatively affect the cell disruption efficiency. However, increasing these parameters positively affect the cost of the cell disintegration process by reducing the

specific energy consumption. The effect of biomass flow rate on specific energy consumption shown in Figure 7. The biomass flow rate is given as DCW influent (kg/h) and the specific energy consumption (kWh/kg) is calculated based on total energy consumed (kWh) to disrupt per kg (in dry basis) of microalgae biomass. One recorded, for an increasing retention time from 1.3 to 2.3 min, an increase of 70% in biomass disruption efficiency and a decrease of 44% in the specific energy consumption. Oppositely, at significantly larger retention times (16 and 28 min), the specific energy consumption increased with 32% because of a lower throughput. The energy consumption of single pass bead milling operation of Chlorella sp. by using a Netzsch, Labstar LS1 recorded as 0.85 kWh/kg dry weight. Similarly, calculated the energy consumption for disrupting Chlorella sp. by a semi continuously operated Dyno-Mill Research Lab as 0.81 kWh/kg dry weight. The recorded values are just 13%-14% of the caloric value of microalgae biomass calculated by (6.083 kWh/kg). In practice, however, the specific energy consumption highly depends on DCW concentration/load, the species and the growth conditions of biomass. Despite many positive characteristics, the high energy demand of bead milling make it less favorable for microalgae biorefineries. The inefficient energy transfer from the rotating shaft to the individual cells and energy conversion into heat require intensive, energy demanding cooling to allow the recovery of functional fragile products (e.g., RuBisCO). Additionally, the formation of very fine cell debris and non-selective distribution of bio-chemicals over the soluble and solid phase result in increased downstream processing costs. Although protein extractabili-ty and digestibility are increased after treatment and the method is effective against microbial and fungal infestations present in the microalgae culture, it is not an ideal disruption method for mild microalgae biorefineries. [13]

Micro-algae	Product	Conditions	Scale	Outcome	Analyses
Bead milling Scenedesmus quadricauda (fresh) Scenedesmus quadricauda (spray dried) Scenedesmus quadricauda (fresh)	Disrupted biomass	Ballotini beads, 33% bead filling, 2800 rpm agitator speed, 5 min, 5% DCW 0.35–0.5 mm beads, 50% bead filling, 1450 rpm agitator speed, 40 l/h flow rate, 5% DCW	1 l grinding chamber, 5 liter grinding chamber	55% cell disintegration 87% cell disintegration 90% cell disintegration	Cell count
Chlorella sp.	Disrupted biomass	7.5 kW, 0.5 mm ZrO_2 beads, 70% beads filling, 15.8% DCW, 62 kg/h feed rate, 90 min 3.3 kW, 0.42–0.58 mm glass beads, 82% beads filling, 10.7% DCW, 3 kg/h feed rate 25 kW, 0.6–0.8 mm ZrO_2 beads, 85% beads filling, 12.4% DCW, 35 kg/h Feed rate 3 kW, 0.3–0.4 mm glass beads, 85% beads filling, 6.9% DCW, 10 kg/h Feed rate, 3000 rpm agitator speed, 2 cycles	1.5 l grinding chamber 1.4 l grinding chamber 18.3 l grinding chamber 0.6 l grinding chamber,	98.5% cell disintegration of Chlorella 99.9% cell disintegration of Chlorella and 90.2% cell disintegration of bacteria 85.29% cell disintegration of Chlorella and 81.2% cell disintegration of bacteria 98–99% cell disintegration of Chlorella and 99.5% cell disintegration of bacteria	Cell count, dry weight
Tetraselmis sp.	Protein	3.3–4 kW, 0.3–0.4–0.6 mm ceramic beads, 65% bead filling, 12% DCW, 1.5 l/min flow rate, 30 min	0.3 l grinding chamber	21% of proteins transferred to algae juice after treatment	Total protein

Table 6. Summary and comparison of case studies on bead milling [13]
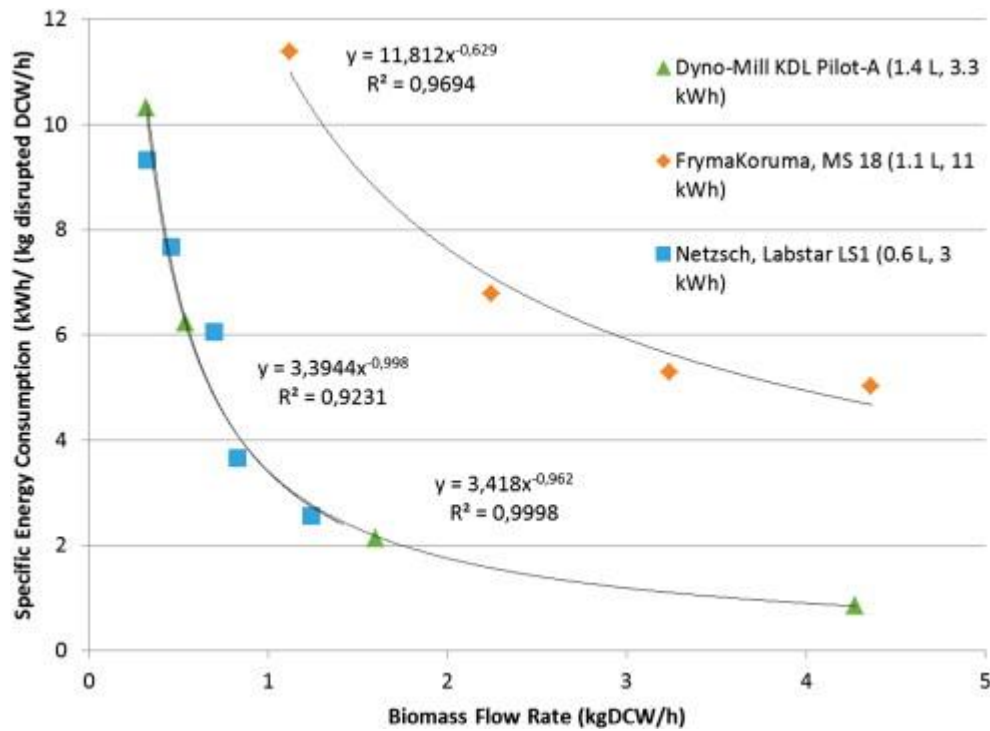
Figure 7. The effect of flowrate on specific energy consumption of bead mill with different equipment, similar chamber volume and different dry cell weight. [13]

2.1.2 High pressure homogenization

High pressure homogenizers (HPHs) are especially suitable for emulsification processes. Various valve-seat configurations are available for HPHs to optimize the disruption efficiency. The cell suspension flows radially across a valve, strikes an impact ring, exits the valve and flows either to a second valve or to a discharge. Cell disruption is thus achieved through high pressure impact (shear forces) of the accelerated fluid jet on the stationary valve surface as well as hydrodynamic cavitation from the pressure drop induced shear stress. Cavitation is defined as a 3-step phenomenon taking place in short time intervals (micro to milliseconds) that starts with the formation of bubbles, followed by growth and ends with the collapse of microbubbles. This causes the release of large amounts of energy into a very small volume. Very high energy densities (energy released per unit volume) are obtained locally which leads to cell disruption. An overview of the case studies is given in Table 7 and discussed below. The literature on HPH shows that a high working pressure followed by the cycle number has the most positive effect on cell disruption efficiency. Lower DCW concentrations and culture stress levels (N-depletion) were significant but to a minor extent and the nozzle diameter was determined as not effective. The specific energy consumption of HPH is highly dependent on DCW concentration, algae species and the growth conditions of biomass. In different studies, the specific energy consumption varies from 0.25 kWh/kg (1% DCW, N-depleted) to 147 kWh/kg (0.85% DCW, no stress). The lowest recorded specific energy consumption is approximately 4.1% of the microalgae biomass' caloric value (6.083 kWh/kg).

Although HPH is, together with bead milling, the most preferred method for the industrial scale cell disruption of microalgae, there are some disadvantages. The main drawback of using HPH in the mild microalgae biorefinery is the use of low dry cell weight concentrations (0.01–0.85% w/w). This increases the energy demand of downstream processing and water footprint due to isolation of products from dilute streams. Also the non-selective intracellular compound release, difficulties to break hard cell walls and the generation of very fine cell debris are among main problems of HPH. Finally, the reduced digestibility of proteins after treatment can indicate that HPH is not a mild technique and thus not suitable for the isolation of fragile functional compounds. [13]

Micro-algae	Product	Conditions	Scale	Outcome	Analyses
Nannochloropsis salina	Anaerobic digestion and biogas from treated biomass	100 bar, 2 passes, 0.875% DCW	35 ml	32.6% increase in biogas production in comparison with untreated biomass	Biogas production
Chlorococcum sp.	Disrupted biomass	850 bar, 0.85% DCW, 4 passes	200 ml	Over 90% cell disintegration, 83% of colony diameter reduction after first pass 146.94 kWh/kg dry biomass energy consumption	Intact cell count, average colony diameter measurement Energy calculations by using the data from
Nannochloropsis oculata	Disrupted biomass, lipid	2760 bars, 0.1% (wet w/w) approx. 0.023–0.035% DCW cell concentration, 4 passes, nitrogen depleted culture	15 ml	67% cell disintegration, 8.5 times more oil extraction than undisrupted algae	Intact cell count, total lipid
Nannochloropsis oculata	Disrupted biomass	2100 bar, 0.15% (wet w/w) approx. 0.015–0.023% DCW, cell concentration, 100 µm Nozzle, 3 passes	15 ml	≈100% cell disintegration	Intact cell count
Nannochloropsis sp.	Protein	1500 bar, 1% DCW cell concentration, 6 passes, nitrogen depleted culture	250 ml	≈91% Protein extraction	Bradford protein analysis

Table 7. Summary and comparison of case studies on high pressure homogenization [13]

2.1.3 High speed homogenization

A high-speed homogenizer (HSH) is a stirring device at high rpm and usually consists of a stator–rotor assembly, preferably made of stainless steel, with a variety in designs of stators and rotors. The effective cell disruption mechanisms are hydrodynamic cavitation, generated by stirring at high rpm, and shear forces at the solid–liquid interphase. When the impeller tip speed reaches a critical value (8500 rpm), hydrodynamic cavitation occurs due to a local pressure decreases nearly down to the vapor pressure of the liquid. Subsequently, as the liquid moves away from the impeller, the liquid pressure restores proportional to the decrease in velocity and the distance from impeller tip and causes the collapse of the cavities. An overview of HSH case studies is given in table 8 and the main characteristics for the mild microalgae biorefinery are discussed below. [13]

High speed homogenization is the most simple, very effective, but aggressive cell disruption method. Advantages are short contact times and the potential to process suspensions with relatively high dry cell weight concentration (2–6% w/w) thus reducing the water footprint and downstream process costs. Additionally, with HSH increased extraction yields of different biochemicals were observed. Unfortunately, the lowest energy consumption is 156.4% of the microalgae biomass' caloric value and protein denaturation due to shear induced local and bulk temperature increase make this method less favorable for mild microalgae biorefinery. [13]

Micro-algae	Product	Conditions	Scale	Outcome	Analyses
Nannochloropsis sp.	Lipid	10,000 rpm for 1 min, 6% DCW	≈16 ml	Wet extraction yield with HSH reached 75.8-78% of dry extraction yield	Total lipid analysis
Nannochloropsis sp.	Lipid	12,000 rpm, 1:50 (g/ml) biomass solvent, 2% DCW	50 ml	%38 ± 2 (w/w) lipid extraction	Total lipid analysis
Phaeodactylm tricornutum	Antioxidant	14,000 rpm, 30 s, 1:1 (v/v) EtOH or MetOH/water solvent 0.12% DCW	5 ml	EtOH: ≈ 30 mg equivalent ascorbic acid/l antioxidant activity	Total intracellular antioxidant determination (ascorbic acid equivalent)
Pavlova lutheri	Antioxidant	14,000 rpm, 30 s, 1:1 (v/v EtOH or MetOH/water solvent; 0.36% DCW	5 ml	Et OH ≈ 22.5 mg equivalent ascorbic acid/l antioxidant activity	Total intercellular antioxidant determination (ascorbic acid equivalent)

Table 8. Summary and comparison of case studies on high speed homogenization. [13]

2.1.4 Ultrasonication

During an ultrasonic treatment, the energy of high frequency acoustic waves initiates a cavitation process and a propagating shock wave forms jet streams in the surrounding medium causing cell disruption by high shear forces. Numerous designs for ultrasonic systems are available for different purposes such as micro/nano emulsion production, cell disruption and product extraction. For bacterial cell disruption, ultrasonic disrupters operating at 20, 40 kHz and 1 MHz are proposed, but nowadays only large scale 18, 20, 24, and 30 kHz ultrasonication devices are in use due to energy consumption concerns. In literature the specific energy consumption ranges from efficient disruption with 0.06 kWh/kg over inefficient disruption with 36.67 kWh/kg to efficient disruption with 100 kWh/kg. The lowest specific energy demand found in literature was provided by a device manufacturer and the only shared parameter of the process was the 15% DCW concentration of microalgae feedstock. To reduce the amount of energy needed for cell disruption, ultrasonic vibration is frequently combined with chemical cell disruption methods. In literature the direct effect of ultrasonication on solubilization and conversion of biochemicals is also studied. The positive effect on soluble chemical oxygen demand, and nutritional value, the insignificant effect of lipid solubilization and conversion to fermentable sugars and negative effect on monodigestion determined by different studies. An overview of case studies is given in table 9. Several forces are behind the mechanism of ultrasonic cell disruption. Ultrasonic vibrations from the emitting tip result in acoustic cavitation that can disrupt cells as discussed in the High pressure homogenization section, but cavitation also results in thermolysis of water around the bubbles forming highly reactive free radicals (H•, HO•, and HOO•) that react with the substances in water. Bubble implosion and fragmentation during acoustic cavitation produce micro-regions of extreme conditions with estimated temperatures as high as 5000 °C and pressures up to 100 MPa. During treatment, the sample temperature can increase significantly with 50 to 90 °C and destroy proteins and other intracellular metabolites. According to the mechanical mechanisms resulting from the intense turbulence associated with liquid circulation currents are more effective on the ultrasonic cell disruption yield than the chemical changes such as the formation of free radicals. The major drawback of ultrasonication of microalgae biomass is the relatively low cell disruption efficiency for some microalgae species and the local and overall heat production. Temperature control during treatment can improve product quality, however, the effectiveness of cell disruption decreases significantly. The possibility of combining ultrasonication with different solvent systems or other disruption methods to increase the efficiency and decrease the energy demand, remains interesting for the mild microalgae biorefinery concept. [13]

Micro-algae	Product	Conditions	Scale	Outcome	Analyses
Stichococcus sp.	Chlorophylla		3 ml	Local heat caused degradation of chlorophylla	Chlorophyll a
Chlorella sp. Scenedesmus dimorphus	Lipid	70 W, 90 s 3 cycles with 5 min bearks 100W 2 min, 2 cycles	15 ml	Lipid recovery 21 wt.% No considerable difference in comparison with methods	Total lipids, dry weight
Chlorella Protothecoides	Lipid			Lipid recovery 10.7 wt.% Considerable difference in comparison with methods	
Botryococcus sp.	Lipid	10 kHz, 5 min 0.5% DCW	100 ml	Lipid recovery 8.8 wt.% Considerable difference in comparison with methods	Total lipid, fatty acid compositon
Chlorella vulgaris				Lipid recovery 8 wt.% No considerable difference in comparison with methods	
Scenedesmus sp.				Lipid recovery 9 wt.% No considerable difference in comparison with methods	
Nannochloropsis salina	Anaerobic digestion and Biogas from treated Biomass	200 W, 45 s, 30 kHz	Analytical, volume not given	21% decrease in biogas production in comparison with untreated biomass	Biogas production
Chlorella vulgaris	Lipid	600 W, 30 s 34 cycles with 5 second breaks	Laboratory (N50 ml), volume not given	5.11 fold more extraction than untreated cells	Total lipid
Chlorella sp.	Lipid	50 kHz, 15 min, 0.5 % DCW	100 ml	2.625 fold more extraction than untreated cells	Total lipid
Nostoc sp.				2.57 fold more extraction than untreated cells	

Tolypothrix sp.	Lipid			3.625 fold more extraction than untreated cells	
Chlorococcu sp.	Disrupted biomass	130 W, 5 min. 5 cycles, 0.85% DCW	200 ml	Nearly no cell disruption =70% of coclony diameter reduction after 3rd cycle 36.67 kWh/kg dry energy consuption	Intact cell count, average colony diameter.
Scenedesmus obliquus	Fermentable sugars	200 W, 30 s 5 cycles with 10 minutes break, 7-10% DCW	5 ml	Complex sugars were converted to fermentable sugars, yield: 0.025 equal gram of glucose/gram biomass.	Total sugars, monosacchari des
Synechocysti s PCC 6803	Lipid	2 kW, 3 min, 52^{0}C outflow temperature, 0.2% DCW 2 kW, 30 s 15 cycles with 30 s break, 26^{0}C , approx. 0.2% DCW	Analytical, volume not given	27.8% (w/w) Lipid release, SCOD increase as much as 29.8% of total COD of biomass 14.77% (w/w) lipid release, SCOD increase as much as 6.7% of total COD of biomass	Total lipid, SCOD analysis

Table 9. Summary and comparison of case studies on ultrasonication [13]

2.1.5 Microwave treatment

Microwave treatment at 2450 MHz is known as the optimal value for heating, drying and cell disruption. When a suspension is exposed to microwaves, the microwaves interact selectively with the dielectric or polar molecules (e.g., water) and cause local heating as a result of frictional forces from inter- and intramolecular movements. The free water concentration in cells contributes to the microwave efficiency for cell disruption. Water exposed to microwaves reaches the boiling point fast resulting in expansion within the cell and an increase in the internal pressure. The local heat and pressure combined with the microwave induced damage to the cell membrane/wall, facilitates the recovery of intracellular metabolites. To distinguish the effect of microwaves from microwave induced temperature increase, the yield of microwave treatment compared to a regular heat treatment at the same temperature and 37.5– 44.4% of total yield determined as related to microwaves. However, since only a fraction of the water is held inside the cells, the majority of the radiation energy is absorbed by the surrounding medium and lost as heat causing protein aggregation and denaturation. [13]

The variations in species and the DCW (0.16– 7.6%) concentrations make a direct comparison of the specific energy consumption impossible. The potential of using high DCW concentrations compared to some other techniques is beneficial for the specific energy consumption. However, since the disruptive effect is mainly based on the absorption of microwave energy by water molecules and subsequently the formation of heat and radicals, it can be derived that the effect of microwave treatment is higher on diluted suspensions in comparison with concentrated suspensions. Advantages of microwave treatment are effectiveness, even for robustness, and easy scaled-up because of the simplicity of the technique. The temperature increase is more homogeneous compared to conventional heating, thus heat related denaturation occurs less readily. Depending on the microalgae species microwave treatment is even more efficient than both ultrasonication and bead milling. Additionally, disruption can be combined with selective extraction (microwave assisted extraction, MAE) which is superior to ultrasonication and microwave heating in terms of speed, efficiency and protection against thermal denaturation. [13]Even though microwave assisted (extraction) processes have the potential to increase the extraction yield and decrease the amount of solvent, there are also numerous problems. The technique is limited to polar solvents and not suitable for volatile target compounds. The formation of free radicals, temperature increase and chemical conversion could interfere with the recuperation of fragile functional compounds making microwave treatment less favorable for mild microalgae biorefinery as a cell disruption method. [13]

2.1.6. Pulsed electric field treatment

Pulsed electric field (PEF) or high intensity electric field pulse (HELP) uses an external electric field to induce a critical electrical potential across the cell membrane/wall. Cell disruption by PEF is caused by electromechanical compression and electric field-induced tension inducing pore formation in the membrane/wall (electroporation). The size and number of the pores is directly related to the electric field strength and pulses. It has been demonstrated that pore formation can be reversible or irreversible. Reversible cell membrane/wall damage occurs if the total area of induced pores is small in comparison to the total surface area of the wall. On the other hand, if the ratio of total pore area to total wall area exceeds a certain limit as a result of a process at relatively higher field strength, the wall is no longer able to repair itself and is irreversibly damaged. PEF does not only destroy the cell wall, but also affects the molecules inside the cells. Though temperature increase is not the mechanism of cell disruption, the increase in bulk temperature during treatment leads to a reduced nutritional value and protein digestibility, the decomposition of fragile compounds and an increased extraction of lipids and proteins. The specific energy demand, calculated with literature data, strongly depends on the concentration of the suspension and ranges from 0.42 kWh/kg for 10% DCW to 239 kWh/kg for 0.03% DCW. An overview of the case studies is given in table 10.

Pulsed electric field can be scaled-up easily and combined with different biomass treatment methods. However, the solution, which will be treated, must be free of ions, i.e., electrically non-conductive, thus limiting the use of this cell disruption method in mild microalgae biorefineries. PEF treatment of marine microalgae would require prewashing and deionization to increase the electrical resistance of the medium surrounding the cells. Additionally, the energy consumption and cell disruption yield vary dramatically related to the medium composition. For example, the increased conductivity associated with the release of compounds from disrupted microalgal cells causes local temperature increases and subsequently a decrease in cell disruption efficiency. The decrease in disruption efficiency due to the release of intercellular compounds makes this technique less suitable for the mild microalgae biorefinery.

Micro-algae	Product	Conditions	Scale	Outcome	Analyses
Pulsed electric field					
Synechocystis PCC 6803	Lipid	59.67–239 kWh/kg, 36–54 °C outflow temperature, 0.03% DCW	Analytical, volume not given	DW loss % 1.37–% 9.54, Reduced solvent need for lipid extraction	Cell viability, Total lipids, Lipid composition
Synechocystis PCC 6803	Lipid	120 kWh/kg, 46 °C outflow temperature, 0.037% DCW 120 kWh/kg, 36 °C outflow temperature, 0.037% DCW	Analytical, volume not given	Extraction similar to untreated cells, SCOD increase 4.9% 1.09 fold more extraction than untreated cells, SCOD 1.4%	Total lipid, SCOD analysis
Nannochloropsis salina	Protein	15.44–30.89 kWh/kg, 37 °C outflow temperature, 0.0545–0.109% DCW	1.08 ml	4 fold more extraction with water than methanol extraction of untreated cells	Bradford total protein, SDS-PAGE
Chlorella vulgaris		2.3 kWh/kg, 37 °C outflow temperature, 0.73% DCW			
Auxenochlorella protothecoides	Lipid	0.42–0.63 kWh/kg, 10% DCW	2.112 ml	Over 3 fold more extraction with ethanol	Water soluble dry dontents, Carbohydrate, Lipids
Ankistrodesmus falcatus	Lipid	5.8 kWh/kg, 0.19% DCW	4 ml	Over 2 fold more extraction with ethyl acetate-methanol	Microscopic investigation, Total lipids, FAME analysis

Table 10. Summary and comparison of case studies on Pulsed Electric Field. [13]

2.2 Non-mechanical methods

Non-mechanical methods often involve cell lysis with chemical agents, enzymes or osmic shock. These methods are perceived as more benign than mechanical processes since cells are often only perforated or permeabilized rather than being shredded. For example, chemical and enzymatic methods rely on selective interaction with the cell wall or membrane components that modifies the cell boundary layer and allows products to leach. An overview of case studies for non-mechanical methods is given in Table 11. [13]

Micro-algae	Product	Conditions	Scale	Outcome	Analyses
Enzymatic treatment					
Chlamydomonas reinhardtii UTEX 90	Dextrin	Thermostable α-amylase 0.005%, 90 °C, 30 min	Laboratory, volume not given	25.21 g/l dextrin	Dextrin
Haematococcus pluvialis	Astaxanthin	0.1% protease K and 0.5% driselase, 1 h, pH 5.8, 30 °C	Laboratory, volume not given	1.65 fold more extraction than untreated cells	Astaxanthin, total carotenoids
Chlorella vulgaris	Lipid	Cellulase, 10 h, pH 4.8, 55 °C, 5 mg/l enzyme Lysozyme, 10 h, 55 °C, 5 mg/l enzyme Snailase, 2 h, 37 °C, 5 mg/l enzyme	Analytical, volume not given	8.1 fold more extraction than untreated cells 7.46 fold more extraction than untreated cells 2.366 fold more extraction than untreated cells	Total lipid
Chlorella pyrenoidosa	Carbohydrates from cellulose, lipids	Cellulase, 24 h, pH 4.6, 50 °C, 140 mg/m^2 enzyme, 2% DCW	15 ml	62% cellulose hydrolysis, 75% increaset in lipid extraction	Total carbohydrates, reducing sugar, immobilized enzyme content, FAME analysis
Chemical treatment					
Haematococcus pluvialis	Astaxanthin	0.1 M HCl, 15–30 min 0.1 M NaOH, 15–30 min	Laboratory, volume not given Laboratory, volume not given	2.65 fold more extraction than untreated cells 1.8–2.2 fold more extraction than untreated cells	Astaxanthin, total carotenoids
Chlorococcum humicola	Fermentable sugars	0.56 M (v/v) H_2SO_4, 160 °C, 15 min	Laboratory, volume not given	Complex sugars were converted to fermentable sugars, 0.52 g ethanol fermentation from treated microalgae biomass	Carbohydrates, ethanol
Chlorococcum infusionum	Fermentable sugars	0.3 M NaOH, 120 °C, 60 min, 5% DCW	Laboratory, 100 ml	Complex sugars were converted to fermentable sugars, 0.26 g ethanol fermentation for per gram treated microalgae biomass	Ethanol, glucose, cell size
Chlorococcum sp.	Fermentable sugars	1.51 M H_2SO_4, 160 °C, 45 min, 0.85% DCW	Laboratory, volume not given	Proteins and pigments were destroyed. Complex sugars were converted to fermentable sugars	Intact cell count, average colony diameter
Scenedesmus obliquus	Fermentable sugars	1 M H_2SO_4, 120 °C, 30 min, 10% DCW	Laboratory, 5 ml	Complex sugars were converted to fermentable sugars, yield: 0.286 equal g of glucose/g biomass	Total sugars, monosaccharides

Table 11.Summary and comparison of case studies on non-mechanical methods of disruption [13]

3. Aim of the design

Since my thesis is focused on one of the mechanical methods, I skip the overview of each non-mechanical treatment technology.

It is not a secret that the bead mill is considered to be the simplest and the most economically feasible solution for algae cell wall disintegration.

Since our faculty is going to deal with the algae cell disruption for the first time, it is reasonable to start with something simple and therefore easily understandable. So that, the issues like maintenance, repair, spare parts availability will not cause serious problems and hence will not unnecessarily consume time.

Due to the logical simplicity of the principles of bead mill, and presence of greatly selected key features as well as the results of experiments, which I reviewed in the chapter 2.1.1. I became absolutely sure, that the bead mill is the best choice for the beginning of disintegration researches.

What I can say by this time is that I will do my best to make the equipment as simple as possible.

It must be tiny and not heavy, consisting of minimal amount of parts. I want it to be mounted on the table for comfortable use. So as I imagine, it should be something like a chamber of volume less than two liters with the agitators inside, mechanical sealing at the entering, then goes radial support, then radial – axial, shaft coupling and an electric drive.

4. Design of laboratory bead mill

4.1. Recommendations for design

The design of my equipment is to be done for laboratory purposes and specific information is not widely available by manufacturers. In this situation the design is going to be implemented according to basic logic of bead mill principles, it will be done in appliance with case studies mentioned above and some recommendations which you can find below in this chapter. The key characteristics I have chosen for this equipment are simplicity and easy maintenance. So let us discuss fundamental demands for the equipment.

Orientation There are two orientations possible: vertical and horizontal (figure 8). My choice is horizontal bead mill. The reason is that this orientation ensures most of the issues, caused by the gravity forces. This includes sedimentation of the media which affects homogeneity and thus the disruption effectiveness. Even this effect is not high, and it occurs also in horizontal mill, unlike the vertical one in horizontal case we have smaller volume/area ratio of both product and beads sediment. Uniform disruption is one of our main goals. Due to construction parameters, horizontal orientation means more reliability of the sealing effect of bottom cover, since the bottom cover is supposed to be demountable for cleaning. As for cleaning, the bead mill is easier to maintain in this position. Easier cleaning means possibility to afford often cleaning, which positively results on product quality and lifetime of the equipment. Gravity forces lead to higher concentration of beads at the bottom of the chamber in comparison to the upper side which makes the chance of the blades there to get stuck and damaged. Also, the choice of horizontal position makes the question "how to mount driving mechanism" easier, because the driving mechanism in this case is not going to be above the equipment. There exist other aspects related to the orientation of the bead mill in space, both of them have their own advantages, however the reasons I talked about determined my choice.

Figure 8. Horizontal and vertical bead mills [14]

4.2. Milling chamber study

The milling chamber study includes planning and recommendations of the proportions and other parameters inside the bead mill chamber.

4.2.1 Bead diameter and particle dimensions

The most important for a good milling effect is the appropriate ratio between the size of the beads and the particle size (figure 9). When scaling the equipment either up or down, manufacturers must always keep in mind that the particle size is a constant value, so for each treated product, there is optimal bead diameter, which remains constant. In my task I do not need to worry about scalability of the equipment, but still to choose the proper size of the beads for algae cell disintegration is a critical issue. The reason for this is that beads that are too small will not be able to "destroy" the particle because of their small bead mass. A small bead will only knock on the particle without breaking it (Figure 9). Further, the bead package could start to act as a filter and accumulate particles and agglomerates in the mill. Consequently, the mill will block. In the opposite situation, if the beads are too large the probability of a crash between the beads and the particles falls drastically (Figure 9). In both cases the milling effect will be poor. [15]

By studying different literatures I concluded that the suitable size in for our purpose is nearly 0.5 millimeters (as you can see in table 6). Typical material for algae disruption is ZrO_2, which of course may be replaced by the bead made of another material with different diameter to satisfy laboratory demands.
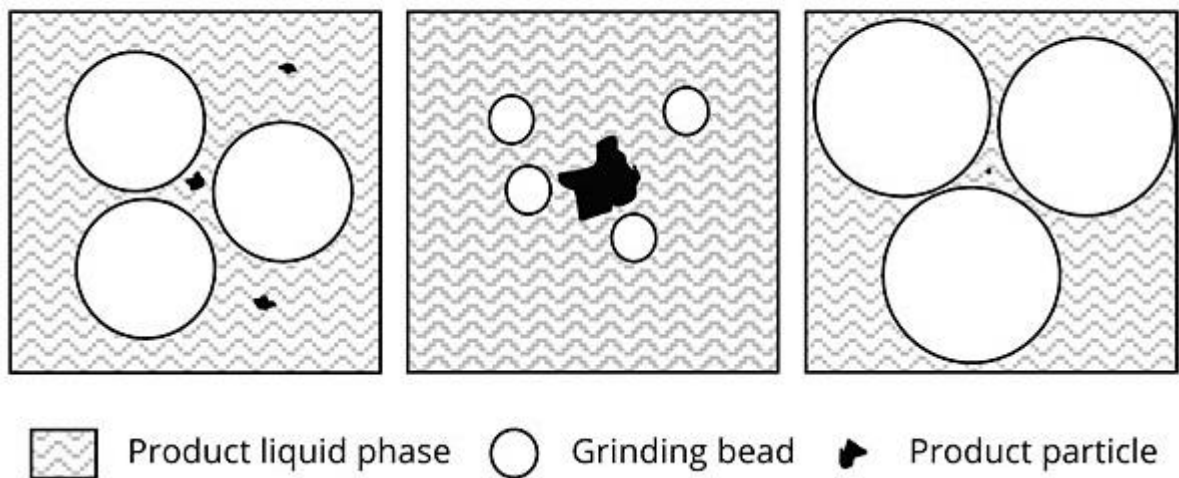
Figure 9. Comparison of correct and wrong bead sizes. [15]

4.2.2 Bead size and milling chamber dimensions

There are also some limits between these parameters. The distance between the edge of the discs and the opposing chamber wall. A minimum rule says that this distance has to be at least three times larger than the diameter of the beads. If we undercut this factor we run the risk of jamming and destroying the beads. [15] Figure 10 shows a large process chamber where this rule is fulfilled, and a scaled down chamber where the situation becomes critical because the rule is no longer fulfilled. Although this figure shows this example on bead mill with pegs, but the rule is also valid for a disc bead mill. Obviously, reducing the bead size is not a solution because the ratio between the particle size and the bead size has to be within the recommended limits.
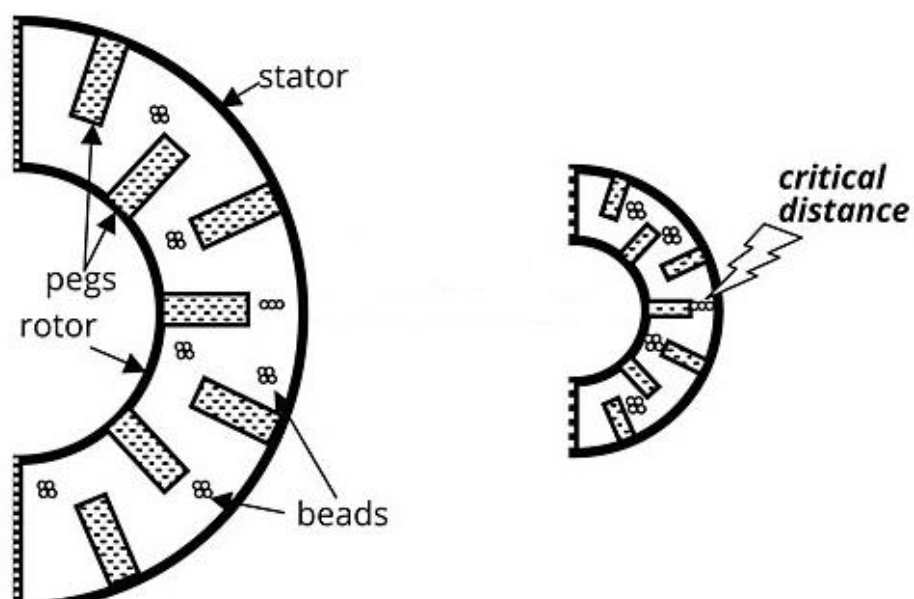
Figure 10. Critical distance between the edge of the pegs and a wall. [15]

4.2.3 Process parameters.

Peripheral velocity or tip speed, is one of the determinative process factor for the outcome product. The choice of the proper speed was an object of many researches. Again according to lots of scientific papers, the recommended value of this parameter ranges in boundary 5 – 14 [m/s] [13] [16] depending on feed rate we want to obtain. E.g. the higher the planned feed rate, the higher the applied tip speed. Since the laboratory equipment does not have the feed rate as a critical property, I focus on reliability and consider the speed 10 [m/s] or below.

Influence of the agitators Most of the variations in the design of bead mill consist of the geometry and installation of the agitator mounted on the shaft. The discs attached to the agitator shaft must be designed so that they give optimal energy transfer to the treated media. Many variations of discs were studied, but eventually there was no attempt to refer disc design to the disruption effectiveness. Discs may be mounted either concentrically or eccentrically, perpendicularly or with incline on the drive shaft. The eccentric setup is supposed to prevent uneven agglomeration of the particles in media. Various disc designs are shown in figure 11. and orientations with respect to shaft in figure 12. Notched or slotted disks are designed to act like a centrifugal pump, imparting movement on the beads. At lower speeds an open agitator design was found to cause greater back-mixing, which reduced efficiency, but at high speeds gave greater agitation and had increased power consumption. Oblique mounting of the impellers on the shaft improved the agitation efficiency at lower speeds but required greater power input and more cooling to maintain the desired operating temperature. [17]
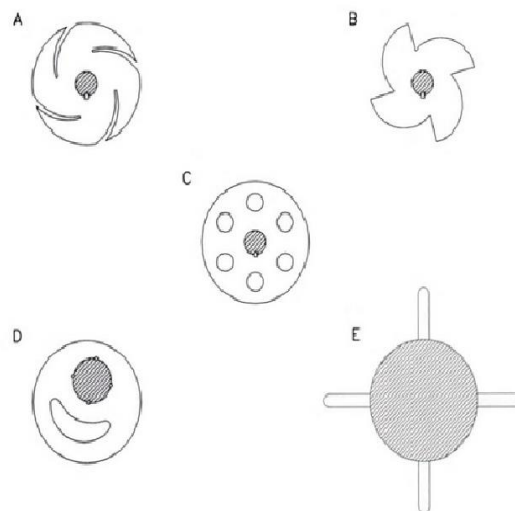
Figure 11. Disc configurations: (A) Slitted disc, closed design; (B) slitted disc, open design; (C) perforated disc; (D) eccentric disc (E) pin agitator. [17]
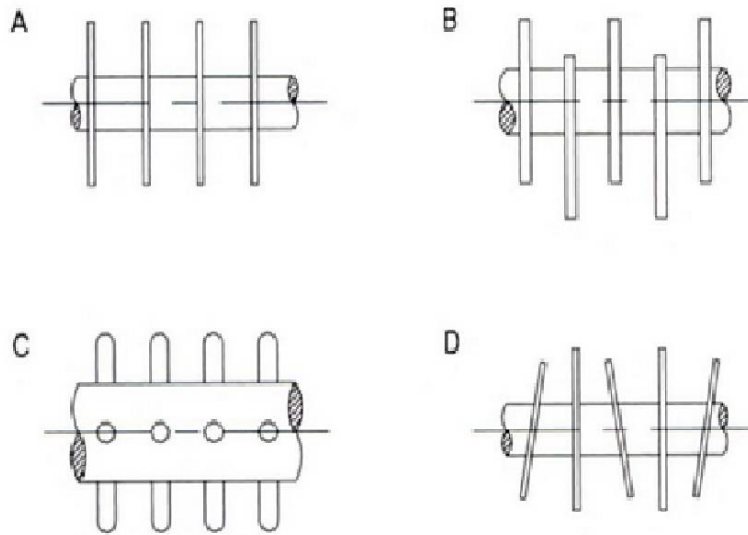
Figure 12. Disc arrangements: (A) concentric, (B) eccentric, (C) pin agitator, (D) oblique(inclined) setup. [17]

That is pretty much it as for the main design parameters which must be considered further.

4.3 Practical design and calculations

This chapter includes necessary strength and dimensions calculations. And discussion of my choices step – by - step.

4.3.1.Preliminary design of general dimensions of the bead mill:

Designing these parameters, I must take into account the summary of case studies. These things you can check again in the table 6. We have decided that the equipment is supposed to be used in the laboratory researches. Therefore the scale of the equipment is the laboratory scale which doesn't need to be bulky and huge. Chlorella sp. as the most widely used type of algae in biofuel production is chosen to be treated. The volume of the chamber is approximately to be more or less 1.5 liters. Proportion of the chamber for better energy transfer was discussed with the supervisor of my thesis and is chosen as H = 1.5D, where H is a height and D is a diameter of the chamber. I want to remind again that I also have to consider the clearance between the agitators and the wall more than three times the diameter of the beads, which by case studies is suitable as 0.5 millimeters. The clearance between the end of the shaft and the bottom has to fulfill the same condition.

Approximate calculation:

$$V = \frac{\pi D^2}{4} * H, \qquad \text{where H = 1.5D} \qquad (1)$$

$$\text{Therefore: } V = \frac{3\pi D^3}{8}, \text{ from that follows: } D = \sqrt[3]{\frac{8V}{3\pi}}$$

By substitution of **0.0015 [m^3]** which makes **1.5 liters** in to the formula above, I obtain the value of the diameter as **0.108 [m]** and round this to **0.1 [m]**.

From which follows: **H = 1.5*0.1 = 0.15 [m]** is the chamber's height.
Now, let's substitute both the height and the diameter to calculate the final volume:

$$V = \frac{\pi D^2}{4} * H = \frac{\pi 0.1^2}{4} * 0.15 = 0.00118 \ [m^3] = 1.2 \ \text{liters}$$
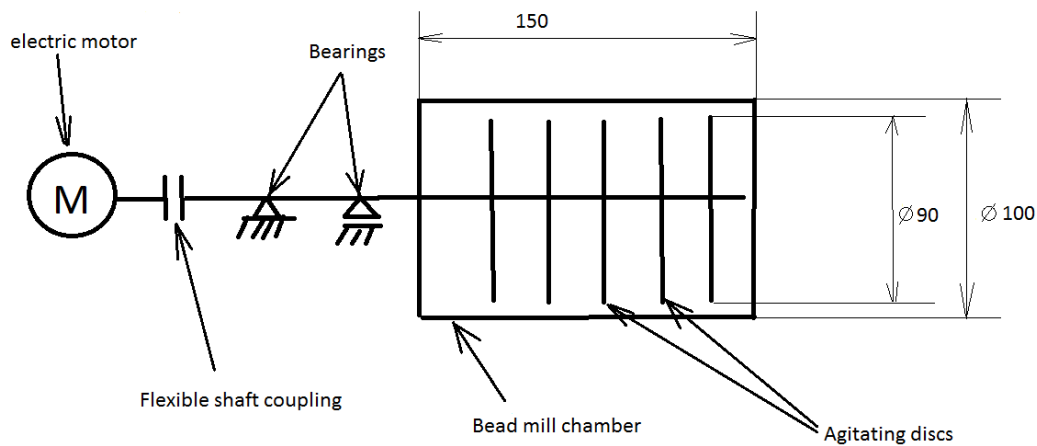
Figure 13. Preliminary sketch with main dimensions

4.3.2 Choice of motor:

The choice of the motor is done in accordance with the case studies. For the volume of the chamber 1.4 liters with feed rate of 3 [kg/h] required power input was 3.3 [kW] table 2. On the other hand, the same source indicates 7.5 [kW] for the volume of 1.5 liters, but in this case the feed rate is 62 [kg/h]. Assuming obtained volume of 1.2 liters and the feed rate around 3 [kg/h], the power input should be a bit more than 3 [kW]. But my opinion in this design involves the principles of reserve capacity. That is why in spite of the fact that the volume is small and the feed rate will probably not be high, I still want to keep some possibility for user to test higher feed rate. Due to this reason I decided to choose the motor with power 4 [kW]. And all the strength check will be calculated according to this power input. In this design the actuator is the electric motor: Nord SK112 L4. It's a three phase, two poles electric motor with the parameters:

Pn……………………………4 [kW]

n………………………..2880 [rpm]

m……………………………26 [kg]

I………………0,0055 $[kg * m^2]$

Flexible shaft coupling is supposed to be used for torque transfer from the motor to the shaft.

4.3.3 Calculation of minimum shaft diameter:

The calculation of the shaft diameter is implemented by VUCHZ calculation procedure. The value on which this method is based is a power input. The only adjustment I have made is related to safety factor. Because this method involves two operation types:

   Light operation: for low-speed agitation with some significant clearance between the agitator and wall, and without any solid particles in suspension. This operation means that if the value of torque is reached to the motor torque times the value of safety factor, then the electric protection is actuated.

   Heavy operation: in case of high speed agitation with relatively high power input takes place, including small clearance, with solid particles in suspension. This takes into account the probability of agitator's jamming.

The rated torque M_{km} is computed from the labeled power input of the electric motor and the operating revolutions per second:

$$M_{km} = \frac{P}{2\pi n} \qquad (2)$$

 Where *P* is a power input of the electric motor, *n* is the number of shaft revolutions per second. By substituting my value I obtain:

$$M_{km} = \frac{4000}{2\pi*35} = 18 \ [Nm]$$

 Where again the required number of revolutions is calculated from the desired peripheral velocity which is 10 [m/s]:

$$n = \frac{v}{\pi d} = \frac{10}{\pi*0.09} = 35 \ [rps] = 2100 \ [rpm] \qquad (3)$$

 Maximum torque, acting on the shaft is calculated from the rated torque value adding the multiplication with safety factor, which depends on the operation type. The VUCHZ calculation procedure involves safety factors:

<div align="center">

k=1.8……for Light Operation

k=2.8…… for Heavy Operation

</div>

 As for design of my equipment, the conditions under which the device operates can somehow share the properties for both types of operations. For example the clearance is assumed to be small which refers to the heavy operation, but the suspension will not contain any solid particles which happen in light operations. The power input in its part is not considered as high, especially due to the fact that the motor will mostly run around 70% of its power. The possibility to block the shaft is dramatically small. After all of these facts it seems to be suitable to assume the safety factor as in light operation. But as I told before, I want this equipment to have some reserve, which means that higher safety than 1.8 is better. After thinking about it, I eventually saw another calculation procedure by EEUA, where the principles and definitions is basically similar, and safety factors are 1.5 and 2.5. My choice was that the

factor 2.5 will easily fulfill the strength conditions, and let me obtain a bit smaller value of minimum shaft diameter than using the factor 2.8.

And finally, the maximum torque, acting on the shaft is:

$$M_k = M_{km} * k = 18*2.5 = 45 \ [Nm] \qquad (4)$$

It is assumed that under conditions of agitators overload and jamming, bending force is acting in a distance of 75% of stirrer diameter:

$$F = \frac{2*4*M_k}{3d} = \frac{2*4*45}{3*0.09} = 1333 \ [N] \qquad (5)$$

And the bending moment it creates is equal:

$$M_0 = Fl = 1333*0.23 = 307 \ [Nm] \qquad (6)$$

Where l is the distance from the closer support to the last agitator at the end of the shaft. By this time I don't know the exact length, but by looking at sketch I choose 0.23 [m]. And my plan is to fit this boundary still having the reserve of few centimeters. Figure 14. Illustrates the forces on the sketch:
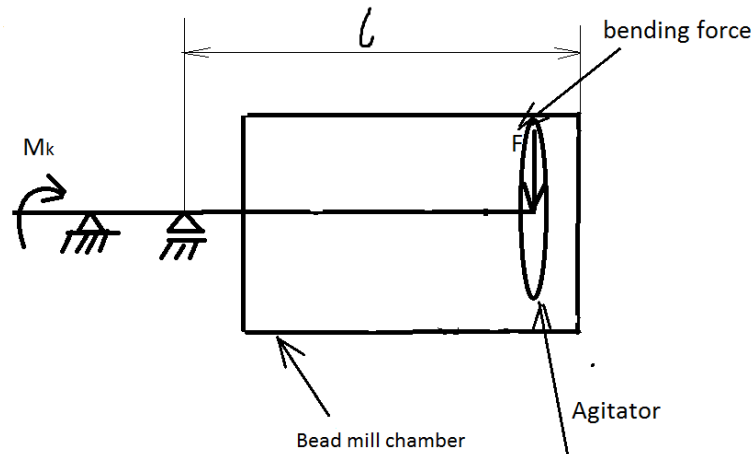
Figure 14. Bending force location

The last thing to calculate is reduced torque:

$$M_{red} = \sqrt{M_0{}^2 + \frac{3}{4}M_k{}^2} = \sqrt{307^2 + \frac{3}{4}45^2} = 309 \ [Nm] \quad (7)$$

Finally the minimum shaft diameter is derived from the ratio between this value and characteristic of yield point of the shaft material:

$$d_0 = \sqrt[3]{\frac{32}{\pi}\frac{M_{red}}{\sigma_{kt}}} = \sqrt[3]{\frac{32}{\pi}*\frac{309}{200}} = 25 \ [mm] \qquad (8)$$

Where σ_{kt} is the yield point. And in my case it is the value for stainless steel with assumption of safety factor equals to 200.8 [MPa].

4.3.4 Wall thickness and temperature control

The specific cooling surface becomes an important issue, but in industrial scale. The temperature in our pearl mill is supposed to be controlled by LAUDA heating/cooling thermostats. Although the tiny bead mill with the laboratory scale doesn't suffer from the temperature problems, it is necessary to provide possibility of some extra cooling. For this purpose I added a double jacket. In terms of better contact and therefore better heat transfer, the inlet and outlet nozzles are mounted so that the first one is located above, while the second one is below. A spiral coil is mounted in the double jacket to make cooling fluid to flow all the way around the chamber before outlet.

Wall thickness is also not the critical parameter, since the chamber is not pressurized. That is why I did not make any strength calculations of the wall. And I have chosen it by looking at the main dimensions to keep reasonable proportions of the equipment. Considering the length around 150 [mm] and the diameter of 100 [mm], the wall thickness from few millimeters at the cylindrical part and up to 10-15 millimeters at the flat plates must be fine.

4.3.5 Agitator discs

As for agitators, I used simple perforated discs Figure 8, mounted on the shaft by means of feather keys and bushing rings between discs. This setup allows us to demount them in order to change one type of agitators to another ones with different shape. By replacing the bushing rings, we will be able to adjust the distance between the discs and consequently their amount. This depends on the disruption conditions and on aims of individual experiments as I mentioned before. So the disc's profile in my drawing is just a formality. Any other shape may be experimentally implemented. The only think is that one will still have to fulfill the clearance conditions, i.e. to have its dimensions more or less the same. The used agitator with respect to design dimensions you can see in the figure 15 and the 3-dimensional view mounted on the shaft is in figure 16.
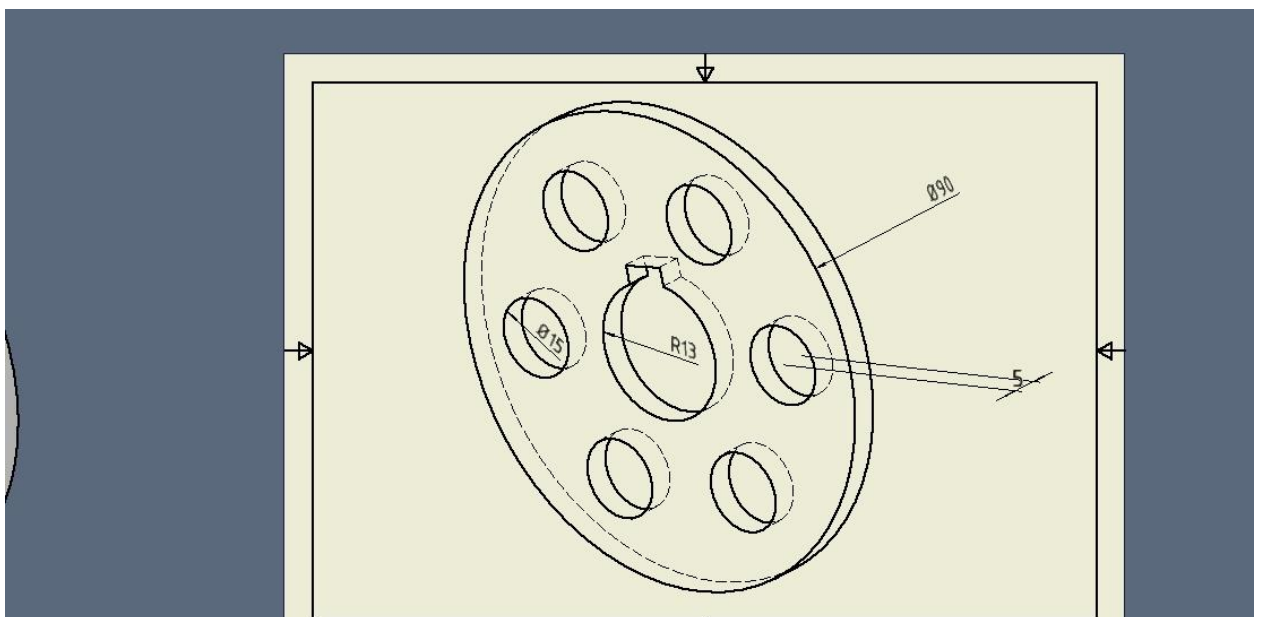
Figure 15. Designed perforated agitator disc with indicated dimensions.

Diameter of disc is 90 [mm] and the diameter of the chamber is 100 [mm] which makes the clearance between the disc and the wall 5 [mm]. Considering bead size of 0.5 [mm] this clearance fulfills the condition to be at least three times greater than the beads' diameter.
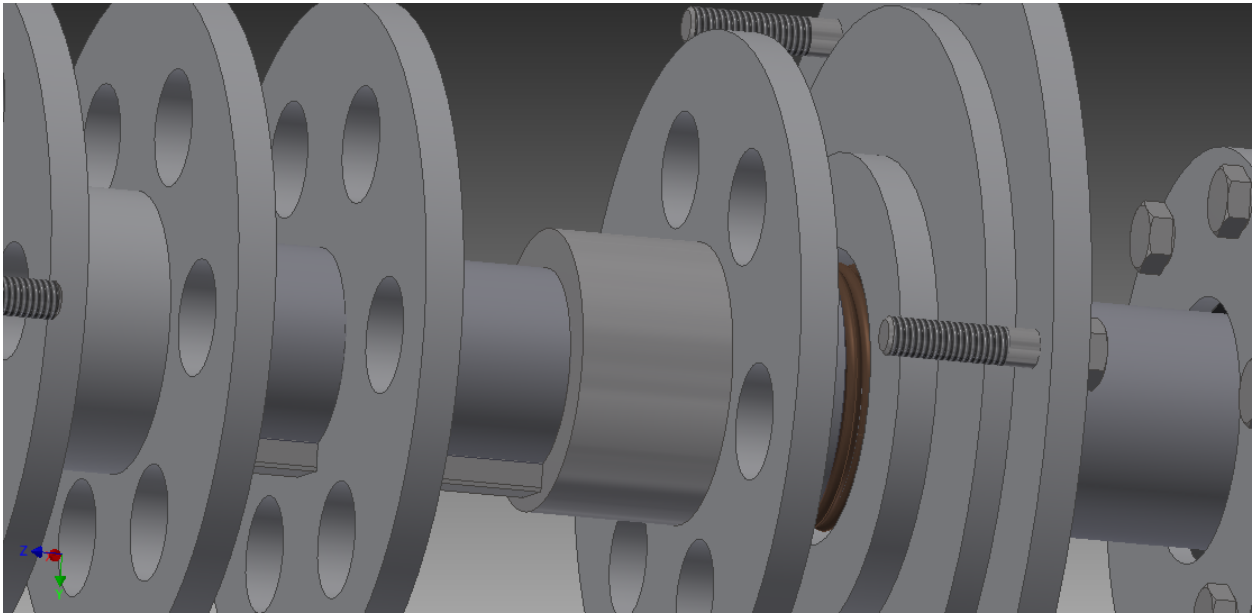
Figure 16. Agitators mounted by means of feather key and bushings.

4.3.6 Sealing:

When we deal with liquid in the chamber, one must provide sealing properties to ensure leakage in such places as lids (cover) connections between the chamber. Also, during the design I must remember about sealing in the bearing housing. But the weakest and the most important place in the equipment is the shaft entering to the chamber. Knowledge, obtained during the course of "Process Equipment Design" with Lukas Kratky, let me conclude that:

Talking about sealing between the chamber and the covers, I just used simple round rubber based insulating gasket (Figure 17).

Figure 17. Rubber gasket seal [18]

As for shaft entering, the task there is more difficult, because we have to avoid axial leakage in movable object. For this purpose, mechanical seal is a great solution (figure 18). You can see general proportions of the dimensions of the mechanical seal with respect to the equipment in the assemble drawing. What one need to note is that the shaft diameter at the entering is 30 millimeters.

For the bearings, sealing lips, mounted into hollows in the covers are conventional solutions. These things are standardized parts, and their standards are indicated in the list of parts.
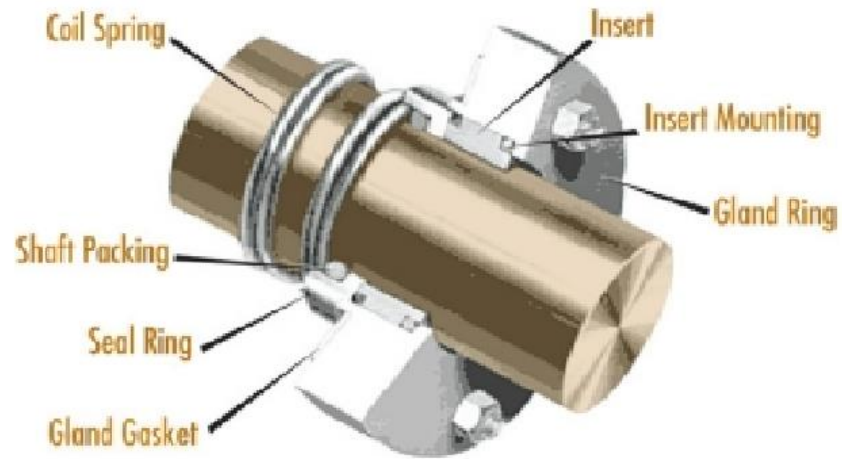
Figure 18. Single mechanical seal [19]

4.3.7 Bearings, their housing and lifetime:

In this design supports are done by means of two single-row ball bearings, mounted in the one bearing housing. The bearing from the chamber's side is an axial bearing, the other which is located closer to the motor's side is fixed to be radial-axial. According to the diameter of my shaft, the bearings with appropriate dimensions were chosen, and from that dimensions of the bearing housing were implemented. All the dimensions see in the drawing. Below, there is a print screen of my design: figure 19.
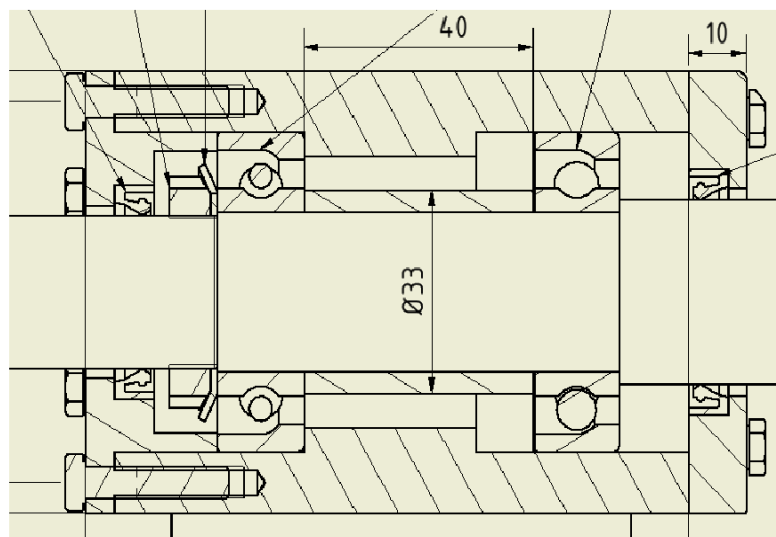
Figure 19. Bearing housing.

As you can see in figure 19, the upper ring of the radial bearing has space for some sliding. The inner ring is fixed by the shaft at the right of it. And the left side of the ring is hold by bushing, which keeps the distance between both bearings. The bushing also holds the inner ring of the radial-axial bearing at the right of it and by means of locknut and a thrust washer mounted on thread on the shaft this ring is fixed from the left side. The upper ring of the radial-axial bearing is fixed by the housing and its cover. Both covers have hollows with sizes, designed to fit the sealing lips. The covers and sealing lips protect the bearings from contamination caused by dust etc.

Lifetime:

To determine the lifetime of the bearings, I have made a stress check based on the radial and axial loads.

Radial load is produced by the reaction of the supports to the bending force caused by electric motor (figure 20):
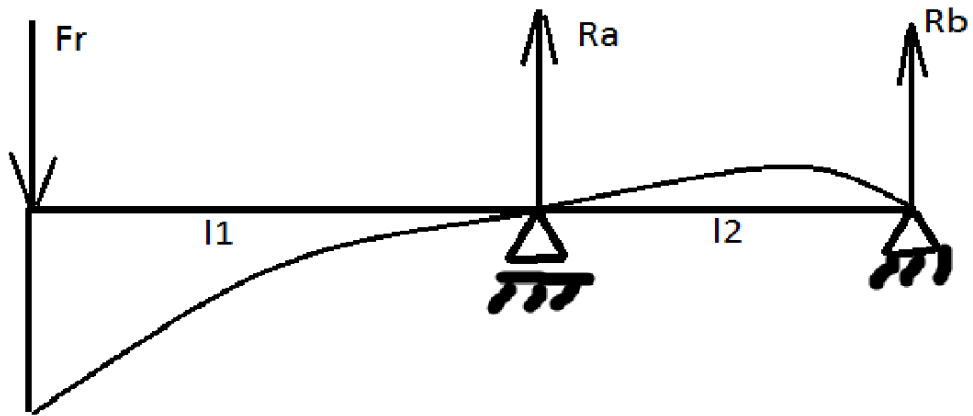
Figure 20. Scheme of moment distribution on the shaft and supports.

And it is equal to:

$$Fr = \frac{2M_k}{d} = \frac{2*18}{0.09} = 400 \ [N] \qquad (9)$$

By calculating simple equilibrium equation we can find both reactions:

$$Fr - Ra - Rb = 0 \qquad\qquad (10)$$

$$F(l1+l2) - Ra*l2 = 0$$

$$Ra = \frac{F*(l1+l2)}{l2} = 1781 \ [N]$$

Where l1 is the distance from last agitator at the end of the shaft and l2 is the distance between bearings: l1 = 190; l2 = 55.

After substitution Ra to the main equation: Rb = |-1381|, i.e. 1381, but the direction is opposite to Ra.

So now we have both radial loads. Let's calculate the axial one. What I we need to assume first is that the axial force acts only on radial – axial bearing. The other support is a sliding support.

Axial load can be estimated as drag force caused by the suspension movement. The drag force is to be calculated from the mass flow of the suspension in the chamber, since the flow moves in the axial direction when comes to the outlet.

These calculations are very rough estimations. Although the drag force caused by such a small flowrate as 3 [kg/h] will generate no practical load, I would like to check it.

So,
$$F_a = F_d = \frac{v^2}{2} * A * C_d , \qquad (11)$$

Where: A is a cross-sectional are of an object (in our case it is the disc), ρ is suspension density, υ is suspension velocity, and C_d is a drag coefficient.

The properties of the suspension which will be treated in the bead mill will be pretty much similar to the properties of the water, since the suspension is mostly based on water (few grams of algae per liter of water). So I can assume the density equals to 1000 [kg/m3].

Agitators' diameter is 0.09 [m], hence cross-sectional area is:
$$A = \frac{\pi d^2}{4} = 0.00636 \; [m^2] \qquad (12)$$

Suspension velocity is possible to compute from the mass flow rate. Assuming the flow rate of 3 [kg/h]:
$$\dot{m} = \rho v A => v = \frac{\dot{m}}{\rho A} = 0.0001 \; [m/s] \qquad (13)$$

Drag coefficient C_d is a function of Reynold's number. To find it, we have to determine the Reynold's number:
$$Re = \frac{v d \rho}{\mu} = 10 \qquad (14)$$

Now, to define the drag coefficient, I used the table, which I found in the book "hydromechanické procesy" 2005 (Rieger F., Novák V., Jirout T.) (Figure 21):

Obr. 6.2. *Závislost součinitele C_D na Reynoldsově čísle Re pro různá tělesa pravidelného tvaru.*
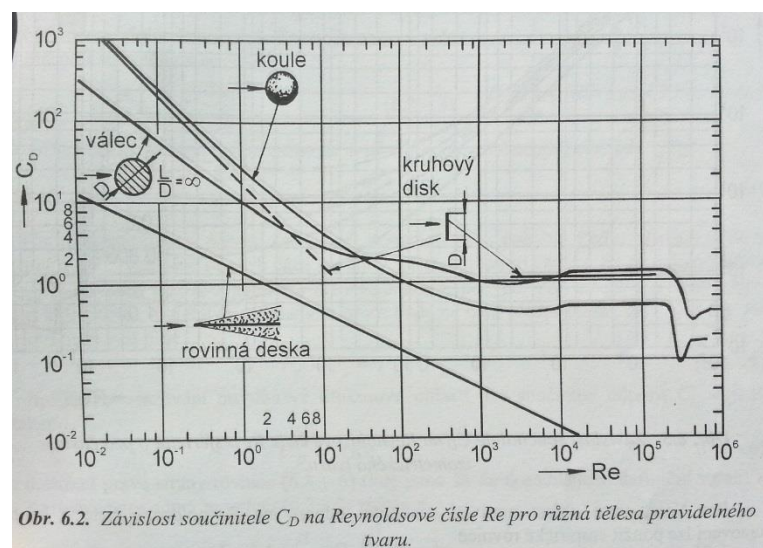
Figure 21. Drag coefficient vs Reynolds number (for basic shapes)

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From the table the value of drag coefficient for Re = 10 is around 1.5.

Now, we can substitute these number to the main equation of the drag force (11):

$$F_a = F_d = 1000 * \frac{0.0001^2}{2} * 0.00636 * 1.5 = 4.77*10^{-8} \text{ [N]}$$

As I told before, the axial force caused by the drag force is literally negligible.

Finally we can calculate the lifetime for the bearings:

Bearing lifetime is defined as:

$$L_h = \frac{10^6}{60n} * (C/P)^3 \qquad\qquad (13)$$

Where n is the number of revolutions per minute, C is a basic dynamic load rating, P is equivalent bearing load, and L_h is the bearing lifetime in hours.

n = 2100 [rpm], C is a bearing parameter given by the manufacturer and in our case is equal 14.8 [kN] The equivalent bearing load P is our radial load.

The result for radial bearing is:

$$L_h = \frac{10^6}{60 * 2100} * \left(\frac{14800}{1781}\right)^3 = 4554 \; hours$$

For axial-radial:

$$L_h = \frac{10^6}{60 * 2100} * \left(\frac{14800}{1381}\right)^3 = 9768 \; hours$$

From the first look, the result for the radial bearing might seem to be insufficient, but this lifetime fits in regular range for workshop equipment of such a famous bearing manufacturer as SKF (figure 22):

Bearing life and load ratings   http://www.skf.com/us/products/bearings-units-housings/roller-bearing...

table 1 - Guideline values of specification life for different types of machine

Machine type	Specification life
	Operating hours
Household machines, agricultural machines, instruments, technical equipment for medical use	300 ... 3 000
Machines used for short periods or intermittently: electric hand tools, lifting tackle in workshops, construction equipment and machines	3 000 ... 8 000

figure 22. Bearing lifetime ratings by SKF.

4.4 Installation:

All parts of the equipment are to be installed on the framework. It consist of few beams with appropriate distances which are welded together. Since the size and weight of construction summarily will not exceed fifty kilograms, the framework with assembled equipment on it, can be mounted on the table. The clearance between the outlet nozzles and the edge of the framework gives us some reserve distance, so that the nozzle hoses and pipelines will not be blocked by the table. As for the nozzles, I did not have an opportunity to mention about sieves. Because the milling chamber is supposed to be filled by beads, which have to remain inside the chamber, all four nozzles have got threads. Necks are screwed on those threads and sieves are mounted inside those neck. That is how we are able to obtain the pure drain without beads in it. The principle is very simple.

 All the necessary dimensions and the standards you can find in the attached drawing and in the list of parts.

The final version of the assembly in 3D is in figure 23. and figure 24:
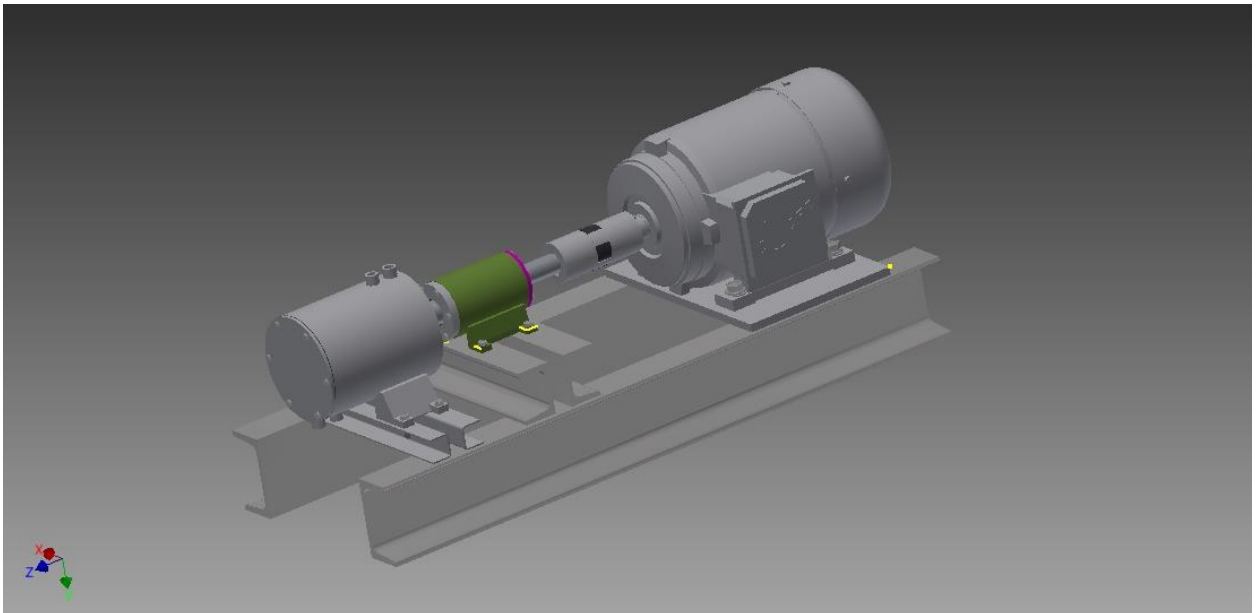
Figure 23. The bead mill assembly.
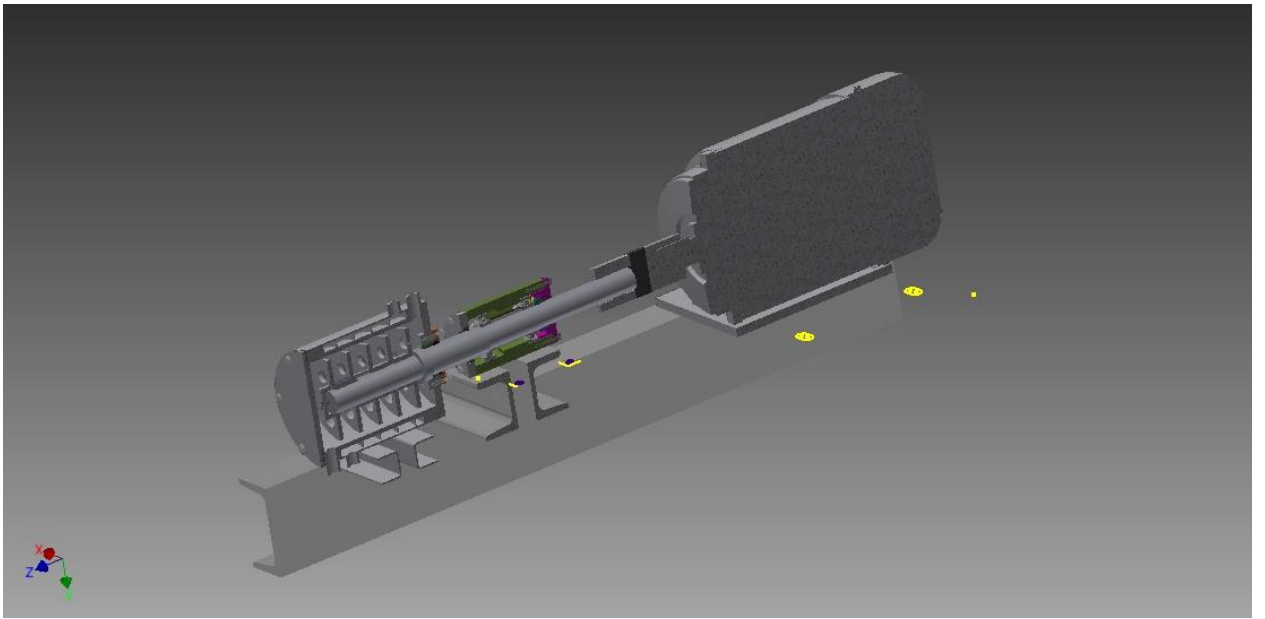
Figure 24. Half section view.

5. Conclusion

- *In this bachelor thesis, the review of the techniques was done with the comparison of the different techniques of cell wall disruption for microalgae biorefineries.*
- *Based on the literature review of the problematics of microalgae's cell wall disintegration, one must admit that because of the high energy demand and high cost of the other technological steps in algae based biofuels production, microalgae biorefineries are not yet feasible, however it does not make this energy source irrelevant.*
- *The comparison of the different techniques, has shown that mechanical treatment (and bead mill particularly) is still more expedient than other modern way of treatment.*
- *The bead mill I have designed is done pretty much close to my primary vision of the plan of design: volume of 1.2 liter, power input of 3-4[kW], 3 [kg/h] feed rate, bead diameter is 0.5 [mm].*

It includes all the required calculations as well as estimations (i.e. strength calculations, process parameters, dimensioning), by scrupulously following of the studied recommendations from books, articles, scientific and industrial sources.

Nothing can be perfect, and there are possibilities to upgrade this equipment. Hopefully, the simplicity of it allows us to make adjustments avoiding huge efforts.

I hope that the design I have elaborated is clearly understandable and everyone who read it will find my work useful and interesting.

I am convinced that the researches in the field of biorefinery will eventually lead to the proper way of natural resources treatment, and I hope that this thesis will be a small, but useful part of these researches.

List of figures

List of tables

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