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17. Harvesting and dewatering of high-productivity bulk microalgae systems (Chapter 17)

Authors

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17.1 Abstract

Autoflocculation, the flocculation of algal cells by precipitation of calcium or magnesium salts from the culture medium, is one promising low-cost method for harvesting and dewatering microalgae for some applications. To date, no single harvesting and dewatering technology currently available is cost-effective for large- scale commercial production of low-value microalgae products, and development of new processes and combinations remains a major technical barrier. The microalgal strain, cultivation methods and culture medium, will determine harvesting, process selection, and efficiency, which may affect biomass quality and the culture medium. The development of a range of viable and controllable harvesting and dewatering systems may help enable the cost-competitive production of bulk microalgae biomass for feed and fuel markets.

17.2 Introduction

Energy-efficient and cost-effective microalgae dewatering, nutrient recycling, and effluent wastewater control are major challenges facing industrial-scale microalgae production for

26 commodity feeds and fuels [1-6]. Due to self-shading, biomass concentrations in microalgal
27 cultures are generally limited from a few hundred milligrams of dry weight per liter in open
28 raceway ponds to a few g L^{-1} in intensive photobioreactors. This necessitates large volumes
29 of water need to be processed to harvest the biomass. For instance, to harvest 1 ton of dry
30 biomass from a relatively dense outdoor pond culture with a 0.5 g L^{-1} dry matter content, 2
31 tonne of culture medium needs to be processed. Filtration through fine screens is not practical
32 as they clog rapidly, while using wider mesh screens, such as microstrainers, is only feasible
33 with filamentous species such as Spirulina (*Arthrospira*). Harvesting microalgal cells from
34 their culture medium by simple gravity sedimentation is generally not feasible in many cases
35 due to the small size of some individual cells, and the small difference in density with the
36 medium. Only larger colonies, clusters or aggregates ('flocs') will readily settle. However,
37 microalgal cell suspensions are stabilized by the negative surface charge of the cells, thus
38 they generally do not spontaneously coagulate to form larger aggregates that sediment rapidly
39 enough by gravity alone. One exception is *Haematococcus pluvialis*, commercially
40 produced for its astaxanthin content, and which can grow as large colonies that settle well
41 (REFERENCE). Another is Pediastrum, a large colonial green alga, which can dominates in
42 high rate algal wastewater treatment ponds (35) .

43 Thus, outside the commercial production of Spirulina and *Haematococcus*, most other
44 larger commercial microalgae biomass production facilities, specifically for Chlorella
45 production, use centrifugation to harvest the biomass, too expensive for all but high-value
46 products (e.g. nutritional supplements). The development and application of microalgae
47 harvesting and dewatering technologies capable of processing large volumes of culture
48 medium at a minimal cost is thus essential for large-scale and low-cost production of lower-
49 costs commodities, such as feeds and fuels (e.g. [1-9]).

50 The objective of harvesting and dewatering is to raise the concentration of the microalgal
51 biomass by more than two orders of magnitude to over 10% solids, sufficiently concentrated
52 for subsequent processing or drying, Most likely, this is best achieved using a combination
53 of technologies in a two stage process [10], such as flocculation followed by centrifugation.
54 For example, the first step would increase the pond culture biomass concentration from
55 about 0.05% (0.5 g L^{-1}) dry matter content to about 2% dry matter slurry, (40 x up-
56 concentration), with centrifugation in the second step, achieving a paste with a 20% dry
57 matter content (a further 10 x up-concentration).
58 Available harvesting and dewatering process selection often interact with both up- and
59 downstream process steps in microalgae production, such as strain selection and medium
60 composition, biomass fractionation (e.g. in a biorefinery) and water or nutrient recycling [11-
61 14]. Performance of any harvesting process will be influenced by properties of microalgae
62 such as cell size, cell fragility, and cell surface charge and reactivity, properties specific to the
63 species and strains, and also influenced by culture conditions [15]. Microalgae can excrete
64 considerable amounts of organic matter in the culture medium [16], particularly when
65 stressed [17], and this may interfere with harvesting and dewatering, and also result in
66 potential contamination.
67 One consideration is that the harvesting-dewatering technology should not impair the
68 quality of microalgal biomass, such as by contamination with coagulants (i.e. alum, iron,
69 organic flocculants). This is particularly important for feed applications [18]. The harvesting
70 method should also allow for recycling of the culture medium in order to reduce total system
71 water demand[19].

72 **17.3** Algal Culture Operations and Productivity

73

74 The main operational modes in microalgal culture are: batch, semi-batch, semi-continuous
75 and continuous. [20]. In batch cultures, a single inoculation (up to 20% of final biomass
76 concentration) is made into a prepared culture system, followed by several days of algal
77 growth until cells reach the desired cell density, followed by harvest of the entire culture.
78 Batch cultivation is used where there is major danger of contamination with invading algae or
79 grazers, etc., and where a high cell density is desired, because centrifugation is used for
80 harvesting. This is the case for commercial *Chlorella* production, where both high cell
81 density is desired and contamination a constant problem. In such a batch process, algal
82 cultures are transferred starting from small laboratory cultures to increasingly larger culture
83 volumes, until the final production pond or photobioreactor is reached, where the culture is
84 allowed to reach maximum density and harvested. Semi-batch would be to retain the same
85 fraction as the original inoculum (e.g. 20%) in the pond, and repeat the batch cultivation, as
86 often as contamination allows. By contrast, in semi-continuous dilution a fraction of the algal
87 pond is harvested daily, the fraction, typically between 20 and 40% of the culture volume,
88 that results in maximum productivity. Continuous cultures are similar except that the culture
89 is constantly diluted and harvested (at least during the daytime). A variant of the continuous
90 cultures is the turbidostat, operating at a fixed density, rather than a fixed dilution . Clearly
91 these operational modes blend into each other and are optimized for balancing productivity
92 (higher for semi-continuous or continuous processes) and harvesting cost (lower for batch
93 and semi-batch cultures. .

94 As an example of these cultivation modes, Moheimani [21, 22] investigated long
95 term growth and productivity of *Dunaliella tertiolecta, Chlorella* sp. and *Tetraselmis suecica*
96 grown in outdoor bag photobioreactors with and without use of external inorganic carbon
97 sources under batch , semi-batch, (harvested once every 7, 5, 3 days), semi-continuous
98 (harvested daily), and continuous (turbidostat) cultures (Figure 1). Changing the harvest

99 regime from batch mode to any of the semi-batch or semicontinuous operations resulted in a
100 significant increase in specific growth rate and biomass productivity for all species.
101 *Tetraselmis suecica* culture achieved the highest specific growth rate and biomass
102 productivity when diluted every day, but lower productivity in continuous culture. *Chlorella*
103 sp. and *D. tertiolecta* showed no difference when harvested every three days or every day
104 These results present the importance of the operational mode and its effect on the overall
105 biomass productivity of the particular microalgae species being cultivated.

106 **17.4** Harvesting of Microalgae:

107

108 After the growth phase, the next step in most cases is to harvest the microalgae; that is
109 to separate the solids (algal biomass) from the liquid medium [23]. The microalgae can be
110 harvested by constraining either the liquid or the biomass, as shown in Figure 2. Liquid
111 constrained (particles are free to move) methods include: flotation (i.e. dispersed or dissolved
112 air), sedimentation and centrifugation. Algae constrained processes, in which the biomass is
113 fixed but the liquid is mobile, include filtration and screening Both types of methods might
114 be implemented at different stages of the harvesting process (Figure 2).

115 Important microalgae properties which can influence their separation are: shape
116 (filamentous, rods, sphere, colonial, etc.); size ; specific gravity ; and charge (usually
117 negative).

118 Due to the small size and low specific gravity of microalgae particles most of solid–
119 liquid separation techniques shown in Figure 2 are not very effective or economical, or both.
120 Separations based on sedimentation or flotation, in particular, are generally improved by the
121 addition of chemical flocculants: positively charged metal salts (e.g. alum, ferric salts) that
122 neutralize the negative charge of the algal cells and bind these into larger aggregates or
123 'flocs' that separate rapidly by gravity [25]. Flocculation followed by sedimentation can also
124 be the result of autoflocculation [26], in which the carbonates in the culture medium act as
125 flocculants, or bioflocculation [25, 27], in which the algae themselves, or, more commonly,
126 associated bacteria, produce or act as flocculants. However, the most commonly used
127 harvesting technology for microalgae, and already applied commercially to wastewater
128 treatment ponds, is chemical flocculation.
129
130 **17.4.1** *Chemical Flocculation*
131 A wide range of inorganic and organic flocculants are used on a large scale in
132 wastewater treatment, drinking water supplies, mining and other industries. Inorganic
133 flocculants include iron and aluminium salts, which, although effective flocculants, are
134 required in relatively high doses, which is both expensive and results in a high content of
135 these flocculants in the biomass as well as the culture medium. Synthetic organic flocculants,
136 such as polyacrylamide–based polymers, are also used in the wastewater treatment, including
137 for separating algae from sewage treatment pond effluents. However the resulting biomass is
138 essentially worthless, as it cannot even be anaerobically digested, and is disposed of. For
139 animal feed applications such flocculants would not be acceptable due to toxicity. Therefore,
140 flocculants based on natural polymers are preferred, with modified natural polymers such as
141 chitosan (de-acetylated chitin) or cationic starch (cationically modified starch) being the best

142 developed and used. However, as for the inorganic metal salts or polyacrylamide polymers,
143 their cost is too high for use in low-cost production of microalgae for feeds or fuels [7, 10].

144 **17.4.2** *Physical flocculation*

145 Standing ultrasound waves have also been used to flocculate microalgae, but this
146 technique is energy-demanding and difficult to upscale [28]. Electroflocculation [29],
147 another process for harvesting microalgae, uses electrical energy to dissolve iron or
148 aluminium ions from an electrode (anode), which then act as flocculants. Due to the high
149 conductivity of seawater, power consumption of electroflocculation is substantially lower in
150 seawater compared to freshwater, making this a potentially attractive method for harvesting
151 marine microalgae [30]. As for other flocculants, electroflocculation results in contamination
152 of the biomass as well as the culture medium, in this case with iron or aluminium salts.

153 **17.4.3** *Bioflocculation*
154

155 Bioflocculation generally refers to flocculation induced by the algae themselves, or by
156 other microorganisms growing with algae or separately principally bacteria. Bacterial
157 flocculation is a well-known in wastewater treatment, and used, for example, in the activated
158 sludge process to remove the biomass produced by gravity settling (sedimentation). Such
159 bacteria can also induce flocculation of microalgae [25], presumably through the production
160 of extracellular polymers that act as flocculants [31]. Bioflocculating bacteria (such as
161 produced in the activated sludge processs) can be cultured separately and added to a
162 microalgae culture to induce flocculation, or consortia of microalgae and bacteria can be
163 cultivated together as so-called micro-algae bacterial flocs [32]. Bacteria mediated
164 bioflocculation-sedimentation is an algae harvesting method in wastewater treatment, where
165 the organic matter supports bacterial growth.

166 In microalgae-based wastewater treatment systems, bioflocculation indeed often
167 occurs spontaneously [33]. Some studies have shown that some fungi may also induce
168 flocculation of microalgal suspensions, plausibly due to positive charges on the fungal
169 hyphae that neutralize the negative charges on the microalgal cell walls [34]. Finally, many
170 microalgae will flocculate spontaneously, due to production of extracellular substances or the
171 formation of larger cell colonies. In high-rate (paddle wheel mixed, raceway) ponds for algal
172 wastewater treatment, sedimentation of the culture is relatively efficient when the microalgal
173 community is dominated by the large species, such as *Pediastrum* [35]. Bioflocculating
174 microalgae may also be used to induce flocculation of a non-flocculating species of
175 microalgae [36].
176
177 **17.4.4** *Autoflocculation*
178 Given that bioflocculation, either with or without bacteria, is typically difficult to
179 control and thus unreliable, and that chemical flocculants are expensive, and also can
180 interfere with further processing or use of the biomass, alternative methods are still needed.
181 One such alternative is autoflocculation – the tendency for algae to flocculate in response to
182 shifts in pH and water hardness. Autoflocculation is due to the co-precipitation of algal cells
183 with Mg^{2+}, Ca^{2+}, carbonates and phosphates, which precipitate at high pH. Seawater, with 52
184 mM Mg^{2+}, 10 mM Ca^{2+}, and 2.2 mM carbonate, will precipitate Mg and Ca carbonates when
185 the pH is raised from roughly pH 8 to above pH 10, because of freshly formed Mg hydroxide
186 and calcite [37-39]. Autoflocculation by calcium phosphate requires both Ca and phosphate
187 and a pH above ~ 8 [40]. A key goal is the engineering of pH, Mg, Ca, and P levels to
188 achieve maximum autoflocculation with minimal chemical inputs. pH rises can be achieved
189 in algal cultures by cutting off the CO_2 supply, letting photosynthesis consume the residual
190 CO_2 and bicarbonate until a high pH is reached, typically in about an hour on a sunny day.

191 Addition of lime can be used to accelerate the process. Understanding better the
192 mechanistic link between pH , precipitate formation and charge neutralization on the algal
193 cell surface should allow the process to be simulated, optimized, and ultimately engineered.
194

195 Engineered algae flocculation is complicated by extracellular organic material
196 secreted by the algae which can aid or hinder flocculation [41, 42]. Algae can also modify
197 their surface charge and their morphology, potentially increasing flocculant demand and/or
198 slowing flocculation (e.g. [43]). Physical factors play an important role, as the rate of alga-
199 alga interactions increase with algae concentration, with shear mixing, and with differential
200 particle settling velocities [44, 45]. Flocculation processes are often species, strain and
201 cultivation conditions specific, such that a flocculant type and dose that work well with one
202 microalgal species or even strain, and cultivation condition, is unsuitable for another. The
203 degree of flocculation also depends on chemical composition of the microalgal growth
204 medium such as ionic environment, temperature, pH.

205 The control of autoflocculation of microalgae would allow for inexpensive
206 dewatering, and could avoid unwanted autoflocculation during the cultivation phase, which
207 could remove both cells and nutrients from the culture. Once the microalgae are flocculated,
208 the microalgae can be separated from the liquid by either simple sedimentation, as discussed
209 her, or : dissolved air floatation or filtration, discussed further below.
210

211 **17.4.5** *Filtration*

212 Filtration requires a pressure differential and a filter media, and can be operated in
213 continuous or batch modes. Filtration is widely used in mining and other industries, and the
214 main advantage of filtration is that most filtration devices are 'off-the-shelf' items. The main
215 filtration methods proposed for microalgae separation include: tangential (cross) flow

filtration [29]; combined gravity belt thickener and dewatering [46]; rotary press [24, 47]; automated filter press [47]; vacuum drum filters [46]; combined screw thickener and dewatering [46], and vibrating screens [48, 49]. The main concerns with filtering microalgae at large scale are the small particle size and the compressibility of the microalgal cells. These properties typically result in filter blockages and fouling, and low biomass recoveries. The most common means to overcome these issues are by using flocculants, filter aids such as diatomaceous earth, or a combination of these. As with any separation technology, filtration is very species-specific.

One filtration technology is limited to very large algal colonies or, in particular filamentious algae, and that is filtration with large mesh (>25 microns) vibrating, inclined and/or rotating backwashed screens (microstrainers). These areused commercially for harvesting *Spirulina*, as these algae are filamentous [49]. However, vibrating screens or microstrainers cannot be used for unicellular or most colonial microalgal species, as a much smaller screen size would be required (and thus low through put).

17.4.6 *Centrifugation* .

Most microalgae can be harvested by centrifugation, which depends on a density difference between the particles and the culture medium. However, due to the low specific gravity of microalgae even centrifugation can be difficult, and very expensive. The various different types of centrifuges include: perforated basket; sieve-scroll; tubular bowl; multi-chamber solid bowl; scroll discharge; disc bowl; nozzle bowl, and decanters [24]. All designs have advantages and disadvantages and to date, decanters are generally the most effective centrifuge for separating most microalgae. The main disadvantages of centrifuges are the high capital costs, operating (power consumption) costs, and, a more minor issue, the effect

on microalgae of associated temperature increases [14]. To overcome or at least ameliorate these issues, centrifugation is often used in combination with other separation techniques. For example, Solix BioSystems in the US worked on combining centrifuges with a patented (by the Los Alamos National Laboratory) algal oil acoustic concentrating and extraction process, with the aim of reducing energy and to avoid chemical solvent extraction [50].

17.5.7 *Sedimentation.*

Gravity separation or sedimentation, was already discussed above in the context of chemical, bacteria–induced or spontaneous algae flocculation methods, and is the simplest and most low–cost method of solid-liquid separation [7, 46]. Sedimentation depends on settling velocity (which can translate into an overflow velocity for the engineering design). Settling velocity is in turn related linearly to the specific gravity difference with the culture medium, and as a square function of the particle size. Thus a ten-fold increase in particle size (e.g. going from single cells to colonies of a hundred cells) increases settling rates one hundred-fold. This allows the relatively rapid settling of larger colonies or flocs, assuming a modicum of specific gravity differences Continuous gravity thickeners and lamellar settlers, are commonly-used sedimentation technologies. Sedimentation separates the microalgae medium into a clear liquid supernatant that can be decanted from the sedimented microalgal slurry (with typically about 1% solids content, though higher concentration factors are reported). The advantages of sedimentation are: lower energy demand, lower capital costs installation costs, and also lower operating skill requirements. The main disadvantages of sedimentation are the low solid content and long settling times required, leading to large space requirements. In the particular case where algae need to go through a flocculation phase before actually settling, larger batch quiescent sedimentation tanks may be required. Sedimentation characteristics are very species and even strain specific and depend on many

factors, including growth conditions but most critically, as noted for cell motility and as noted above, colony or floc size. Due to unpredictable and uncertain control over these factors, sedimentation methods will likely require chemical flocculation as a backup, to maintain the overflow velocity design specifications at all times. For feed applications, it would be necessary to use a feed compatible flocculant, for example chitosan, which may be economically feasible if used in modests amounts in back-up mode. Sedimentation using inclined settlers is an alternative to settling tanks [51].

17.5.6 *Magnetic Separation*

Removal of suspended particles such as microalgae is possible by using magnetic particles such as Fe_3O_4. The coagulated particles can then be passed through a magnetic field leaving the product water relatively microalgae free. Bitton et al [52] reported microalgal removal efficiencies between 55 and 94% from five Florida Lakes by use of a commercial magnetic filter. There is no recent costing available for magnetic separation of microalgae, although Mitchell et al., [53] estimated a capital cost of $545,000 and maintenance and overhead of $0.07 kL^{-1} of treated capacity, and an operating costs for a 56.8 ML d^{-1} treatment plant of $0.01 kL^{-1} for removal of suspended solids from blast furnace scrubber water from steel mills.

17.5.5 *Flotation*

Flotation had its beginning in mineral (ore) and wastewater processing and has a long history in solid/ liquid separation applications using stable froths to selectively separate different minerals from each other [54]. Where the small size of algae makes their recovery from media very difficult, even by centrifugation, an improvement can often be achieved by flocculation followed by flotation. Some microalgal species have buoyancy (due to regulation

of gas vacuoles or high lipid contents) and can naturally float. In other species, if a sufficiently dense floc does not form, assisted flotation techniques can be used, in which small gas bubbles are attached to the flocs, with the separation dependent on the size of the gas bubbles. Large air bubbles can be continuously pumped into a flotation cell for so-called froth flotation, though froth flotation generally has a low recovery efficiency. Dissolved air flotation (DAF) units were developed that injected air-supersaturated water under pressure into the flotation cells, with the floating algae skimmed from the surface. The higher density and smaller footprint of this system, has resulted in full scale installations at several wastewater treatment ponds. Some studies suggest the possibility of bioflotation whereby the photosynthetically generated oxygen would be utilised, however, this is yet to be tested in large scale. The interaction between microalgae and air bubbles depends on the hydrophobicity of the cell surface. Hydrophobicity of cells is typically low, but can be enhanced by addition of surfactants (e.g. [55]). Flotation can be used as an alternative to sedimentation for separating flocculated microalgae from the medium, and, as noted just previously, often yields a higher concentration factor than sedimentation [56].

17.6 Multi-staged Separation

Biomass concentration in microalgal cultures ranges from less than 0.5 g L^{-1} in open raceway pond systems to 5 g L^{-1} in photobioreactors, compared to >50 g L^{-1} for industrial fermentations. Harvesting requires 40-400 up-concentration of the biomass from a dilute culture with 0.05-0.5% dry matter content to a microalgal paste with a dry matter content of about 20%, which would need to be processed immediately, or dried into a stable product. To reduce the cost and energy inputs, a multi-stage harvesting process is proposed [7]. A multi-stage process for harvesting microalgae from a dilute raceway pond culture could include the following stages: primary concentration: ranging from 0.05-0.5 g L^{-1} to 5 g L^{-1}; thickening:

316 from 5 g L^{-1} to 50 g L^{-1} (a thick slurry); final dewatering from 50 g L^{-1} to 200 g L^{-1} (a
317 microalgal paste in which all extracellular water is removed), and drying to 10-15% water
318 content (as needed). In such a multi-stage harvesting process, the recovery efficiency and
319 concentration factor of each stage in the process are key parameters [57]. The recovery
320 efficiency is the proportion of the biomass in the suspension that can be recovered during a
321 harvesting stage. The concentration factor is the ratio of the biomass concentration in the
322 original suspension and in the final concentrate obtained in a harvesting stage. As a multi-
323 stage harvesting will generally require more time than a single-step harvesting process, care
324 should be taken that the biomass quality does not deteriorate. Due to the high metabolic rate
325 of microalgal cells, but more importantly the associated bacteria, this can occur rapidly. As
326 90% or more of water is removed during the primary concentration stage, large energy
327 savings can be achieved by being able to use for primary concentration, gravity
328 sedimentation, flotation, or autoflocculation. Inclined settlers or lamella separators have
329 much shorter settling distances and a larger surface area for collecting settled particles, and
330 are therefore more efficient than gravity thickeners. When conventional inclined settlers are
331 used, coagulation and flocculation is required to increase the particle size and enhance the
332 settling rate of the microalgal cells. If the distance between the lamellae is very small (mm
333 scale), inclined settlers can be used to harvest cells without the need of a coagulant (Smith &
334 Davis 2013).

335 During thickening of the microalgal suspension to a thick slurry the biomass
336 concentration reaches several tens of g L^{-1}, and the viscosity may increase and the biomass
337 slurry may start to display non-Newtonian behaviour such as shear thinning [58]. This may
338 be important when the biomass slurry is to be transferred to the next harvesting stage by
339 means of pumping. The final dewatering prior to drying, if any, is best achieved using a
340 mechanical method such as centrifugation or belt filtration. While these are energy-intensive

341 technologies, the volumes that need to be processed are generally relatively low at this stage.
342 Due to the high content of intracellular water of the harvested biomass, residual moisture
343 even after centrifugation will be 80-85%, requiring removal of between about 4-6 kg of water
344 to produce 1 kg of algal biomass (with a 10% residual moisture). Just to evaporate water
345 requires 2.5 MJ/kg, thus it would theoretically require about 10-15 MJ/kg of to dry algae. In
346 practice it would likely be 30 to 50% more, depending on efficiency, as well as ancillary
347 electricity requirements. Drying could easily consume all the energy that could be
348 recovered as fuel from the algae.

349 ## **17.7** Conclusion
350

351 Cost-effective and non-destructive microalgae harvesting and dewatering techniques
352 are a major operating consideration, and a major challenge to the industry at this time [59,
353 60]. Dewatering options will need to be customized for selected species of microalgae to be
354 mass cultured, and for each application (e.g. high value products, feeds, or biofuels). High
355 productivities are required alongside efficient harvesting and dewatering technologies, to
356 reduce total algal product cost [12, 19, 60, 61]. The development of a range of commercially
357 viable and sustainable algal production, harvesting, and dewatering processes will be required
358 to supply mass-produced microalgae in a shorter time-frame [2, 14, 19, 60, 64]. The final
359 commercial bioproduct production streams and associated costs will be heavily dependent on
360 the species, product, and final use [19], with industrial production system integration likely
361 required to enable cost-effective competitive production of bulk microalgal biomass
362 production across a range of final markets [2, 60, 61, 63-65].

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547 List of Figures:

548 Figure 1. Effect of different mode of dilution on specific growth rate, biomass productivity
549 and photosynthesis parameters of *T. suecica*, *Chlorella* sp. and *D. tertiolecta* (Data are mean±
550 SE, n=4, 7, 18, 36, 73 and 73 for cultures grown under batch, diluted every 7 days, 5 days, 3
551 days, 1 day or continuously).
552

553 Figure 2. Classification of common industrial solid-liquid separation techniques (modified
554 and redrawn from [46].

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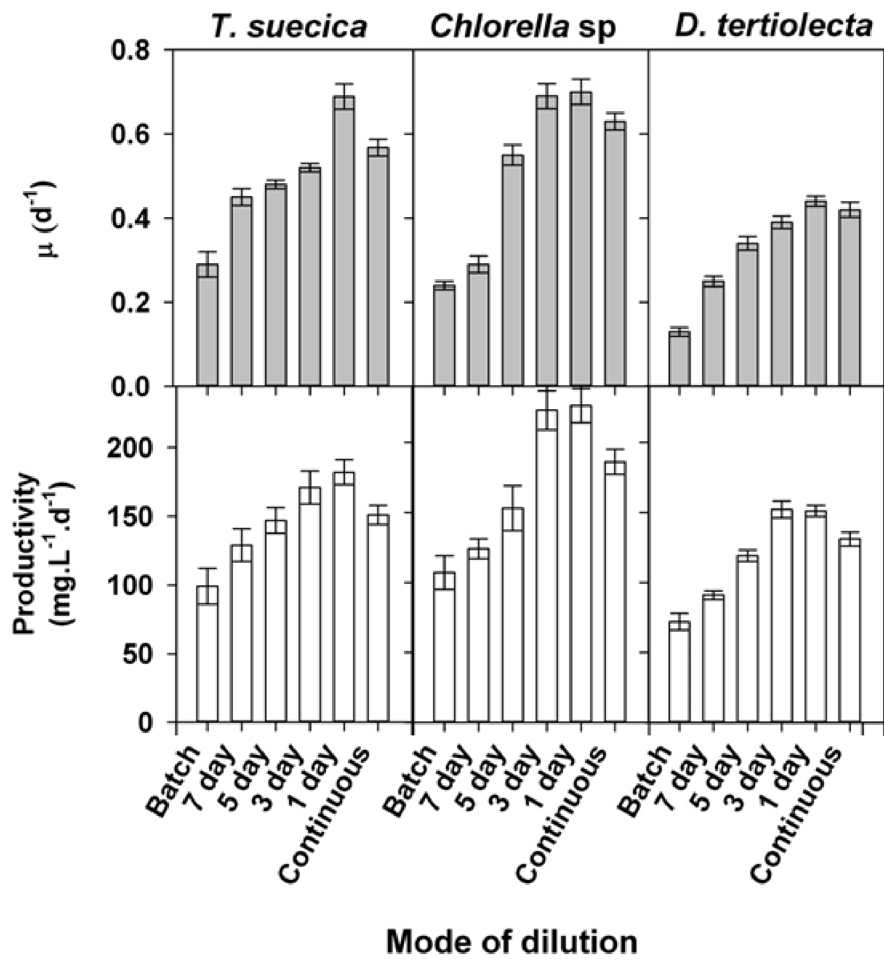
556

557 Figure 1.



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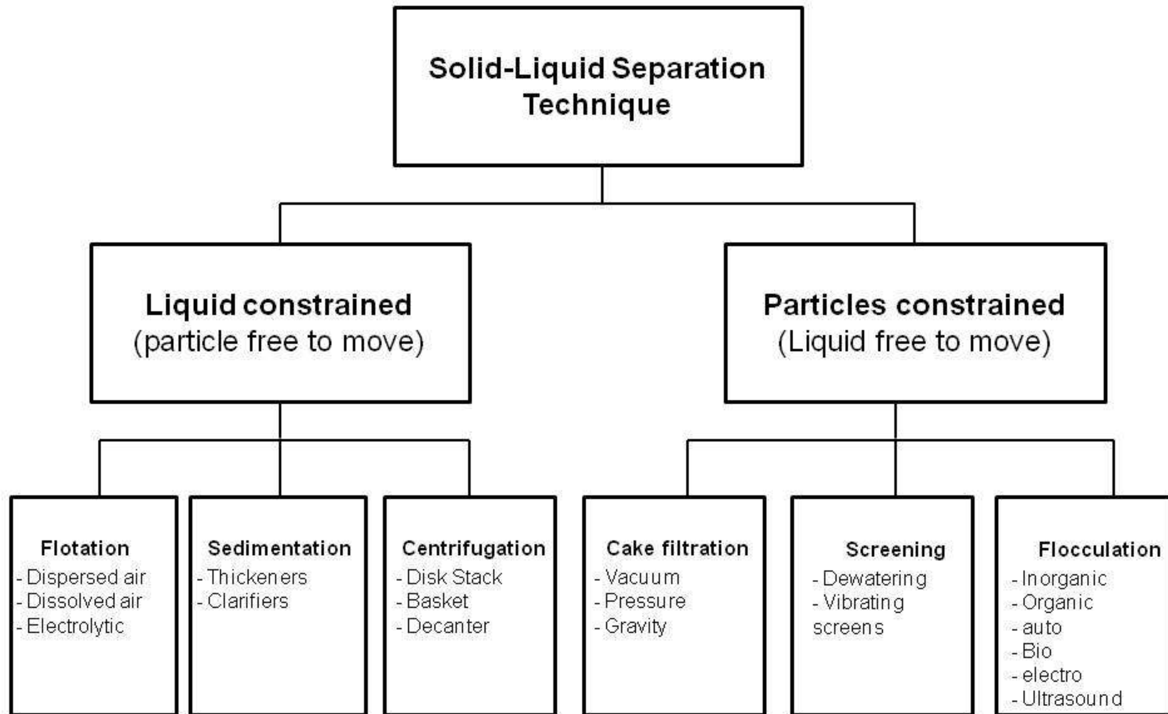

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562 Figure 2.

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