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## **Innovative harvesting processes for microalgae biomass production: a perspective from patent literature**

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**Abstract**

The harvesting of microalgae for biofuel production consists of a primary concentration step, followed by a separation step to isolate the microalgal biomass from its aquatic environment. Recent research focussed mainly on the technological feasibility of various separation processes. However, to what extent these innovative harvesting strategies have been commercialized and therefore have led to actual innovation in the current microalgae biotech industry by the creation of intellectual property, has remained unexplored. This study reviews the scientific literature based on technological, economical and environmental criteria of 13 primary and 8 secondary harvesting methods. Commercial deployment was evaluated via patent analysis. Auto- and co-flocculation, as well as sedimentation, overall scored best for economical (CAPEX and OPEX) and environmental (energy and GHG) criteria, while belt filters scored the highest on the technological criteria (TSS). Hence, only 4 patents based on auto-/co-flocculation, sedimentation and only two for belt filtration are still in force. Technologies based on organic, electrolytic and magnetic flocculation seems to be more successfully patented. Since patenting involves making the technology freely available for others, small but sometimes crucial improvements in low-tech systems may be often kept as a company secret instead. So far, no single harvesting process with superior feasibility has emerged for application on a large commercial scale. This is mainly due to the difference in relative importance of technological, economical and environmental criteria for each harvesting process dependent on the used strain and the final products.

**Keywords: microalgae, harvesting, dewatering, concentration, IP, biofuels**

## 1. Introduction

Microalgae biomass production has gained increasing global interest in the search for renewable resources for a sustainable, bio-based economy. Microalgae are considered as the most promising feedstock for biofuels, but it will still take several years to develop production processes that are both sustainable and economical [1]. Meanwhile, alternative high-value products derived from multiple microalgal components, are further explored ([2]; [3]).

Microalgae can be grown onto non-fertile soils in ponds or photo-bioreactors, in marine or brackish waters using N and P from wastewater resources. Over the last decade, substantial research efforts have resulted in increased microalgal biomass productivity. However, because of ineffective water and nutrient recycling combined with energy-intensive harvesting, the production of microalgal biofuels is currently not competitive with fossil fuels ([4]; [5]).

Because microalgae are small and grow at low concentration in culture, biomass harvesting by conventional separation processes is expensive, which hampers economical microalgal biomass production on a commodity scale ([6]; [7]).

Microalgal harvesting consists of a concentration and a separation process to produce an algal cake, paste or sludge of 15 to 25% or more dry solids from a dilute biomass of 0.02–0.06% dry solids. Harvesting is often divided in primary and secondary concentration steps. Primary concentration methods assist in thickening of the microalgal biomass slurry up to 1–5% in order to facilitate the separation from their culture medium. Further dewatering of the biomass requires an additional step, generally referred to as secondary concentration. This concentration step can

produce a microalgal sludge with an average concentration of about 200 g L<sup>-1</sup>. Generally, concentration techniques are based on physical, chemical or biological processes. Physical concentration techniques apply mechanical or electrical forces to concentrate the microalgal biomass. Ultrasonic waves and electrolysis are used to destabilize the microalgal cells ([8]; [9]; [10]). Chemical techniques make use of inorganic or organic additives to enhance coagulation, or for example (nano)particles with magnetic properties to neutralize the microalgal negative charge for coagulation [11]. Finally, concentration techniques that are based on biological processes to induce spontaneous or natural flocculation, are generally referred to as bioflocculation [12]. These methods do not require additional chemicals but rely on interactions with bacteria, fungi or even with other microalgae species for co-flocculation. Usually, these processes are followed by a secondary dewatering step based on filtration, enhanced sedimentation, centrifugation or flotation [6]. The technological feasibility of most of these separation processes for harvesting microalgae has been experimentally validated in several original studies, and reviewed extensively in technical overviews and techno-economic analyses ([7]; [13]; [14]). However, it is currently not well documented to what extent these novel harvesting strategies have been commercialized and therefore have led to actual innovation in the current microalgae biotech industry by the creation and maintenance of intellectual property.

The aim of this study was to provide an overview of harvesting technologies for microalgae biomass production that have been patented worldwide over the last years. First, a detailed overview of several technological and economical parameters for several harvesting methodologies is given based on scientific literature, followed by a detailed patent analysis to overview currently expired and protected microalgal harvesting processes.

## 2. Materials and Methods

### A. Literature review

The 21 most studied harvesting techniques were selected and compared, i.e. 13 primary concentration/separation and 8 secondary concentration/separation techniques based on technological criteria (strain limitation and final total suspended solids concentration (TSS)), economical criteria (capital expenditures (CAPEX) and operational expenditures (OPEX)), and environmental criteria (energy demand and greenhouse gas emission (GHG)).

The scientific literature was screened in order to obtain information about the final biomass concentration after separation, expressed as total solid suspension (TSS). A high TSS means that the harvesting technology is efficient in terms of concentration, consequently leading to high water removal. The capital expenditure (CAPEX) is the capital investment in equipment while the operational expenditure (OPEX) represents the operational cost. These costs were (a) obtained directly from scientific studies or (b) determined by a relative comparison with other harvesting techniques. For harvesting by disk stack centrifugation, dissolved air flotation, electrolytic flocculation, bioflocculation and sedimentation (c) both CAPEX and OPEX were calculated based on the following equation (1), which represents the total harvesting costs ( $P_c$ ) for the production of 1 m<sup>3</sup> ([15]; [16]):

$$P_c = \frac{((0.5 \cdot I/100 + M/100) \cdot C \cdot A) + C}{W \cdot A \cdot Q_c} + \frac{R_c}{Q_c} \quad (1).$$

Wherein  $I$  = interest rate (% of investment): 6%,  $M$  = maintenance cost (% of investment): 2%,  $C$  = investment cost (EUR),  $A$  = amortization (years); which is the number of years that someone has to pay off for the investment: 10,  $W$  = working hours in a year (h): 8400,  $Q_c$  = capacity ( $m^3 h^{-1}$ ),  $R_c$  = running cost of the system (raw material + energy consumption (EUR  $h^{-1}$ )). CAPEX was calculated by eliminating  $M$  and  $R_c/Q_c$  in the equation.

The energy consumption (energy) is the energy in kWh required to achieve the given biomass concentration per  $m^3$ . The amount of greenhouse gas emissions (GHG) for each method was expressed as the amount of produced  $CO_2$  per required energy unit ( $g CO_2\text{-eq MJ}^{-1}$ ). When no direct data were available, conversion was based on literature studies with the amounts  $CO_2$  produced in relation to the distance ( $g CO_2\text{-eq } 100 km^{-1}$ ), and converted using the formulae:

$$\frac{\frac{10 \text{ kg } CO_2}{100 \text{ km}}}{\frac{1 \text{ MJ}}{0.39 \text{ km}} \cdot \frac{1 \text{ kg}}{1000 \text{ g}}} [17] \text{ or by dividing the reported amounts kg } CO_2\text{-eq ton}^{-1} \text{ algae by the average}$$

microalgal net calorific value ( $18.5 \text{ MJ kg}^{-1}$ ) ([18]; [19]). These types of conversions were applied for decanters ([18]; [19]) and belt filters ([18]; [20]). Other conversions were based on the reported amount of  $CO_2$  emissions per ton biodiesel for disk stack centrifugation ([21]; [20]; [22]), decanters ([18]; [19]), chamber filters ([21]; [19]), inorganic flocculation ([23]; [24]; [25]) and organic flocculation ([26]; [24]). This approach allowed to estimate the amount of greenhouse gas emissions based on literature data across several studies and report it as an interval between minimum and maximum reported values.

### *B. Patent analysis*

Patents were retrieved from the EPODOC database of the European Patent Office (EPO). Only



European (EP), Patent Cooperation Treaty (PCT) and United States (US) patents or patent applications for harvesting techniques, published from 2000 onwards, were retained. The patent search strategy was based on a combination of the International Patent Classifications (IPC), Cooperative Patent Classifications (CPC) or European Patent Classifications (EC) (which is no longer in use) and English, French or German keywords with the boolean operators “OR” and “AND” in full-text EP, PCT or US patent documents. The selected patents were analyzed by six quality indicators, using Espacenet (<http://worldwide.espacenet.com>), the European Patent Register (<https://www.epo.org/searching/free/register.html>) and PAIR (Patent Application Information Retrieval) (<http://portal.uspto.gov/pair/PublicPair>) of the USPTO. Patents that met the quality indicators were discussed in further detail.

#### *i) Search queries*

The first query comprised the keywords: (microalg+ OR algae OR algen+ OR algue+ OR phytoplankton+ OR cyanobacter+ OR algal+ OR biomass).

The second query consisted of: (microorgan+ OR mikroorgan+ OR (mi?ro W organ+) OR cell? OR zell? OR cellule?) AND (biodiesel? OR biofuel? OR biobrennstoff+ OR biokraftstoff+ OR biocarburant+ OR biocombust+).

Both queries were introduced in combination with 91 different IPC, CPC or EC (actually replaced by CPC), representing the 21 harvesting techniques.

#### *ii) Patent quality indicators*

Patent selection was based on the following quality indicators: (1) grant of a patent application, (2) payment of renewal fees, (3) patent family size, (4) number of International Patent Classifications (IPC), (5) number of backwards citations cited in the international search reports and (6) number of claims. These indicators [27] were adapted to measure the relative impact of the retrieved patents or applications.

A granted patent (1) means that the application met the patentability conditions, i.e. novelty, inventive step and industrial applicability. However, a patent application that is not yet granted, but still under examination and which is thus not abandoned or withdrawn, will also be taken in account. Secondly, patents for which the renewal fees were paid for at least 5 years or at least the first annual fee (= in the 3.5<sup>th</sup> year) for the US (2) were also retained. The family size (3) is the number of equivalents filed for an invention in different countries, based on one or more earlier priority applications. ‘Many family members’ means that multiple patents are filed in several countries. Patents with at least one other family member were selected.

Patents with at least 3 IPC (4) were subsequently retained. A large IPC number means that the invention can have a wide number of technical applications. The number of backward citations (5) is another indicator that relates to the number of prior art documents. A small number of backward citations means that the technology could be a pioneer for that technical field. The number of “X” (=novelty) or “Y” (=inventive step) documents cited in the search report are indicators of the importance of the invention against the background prior art. A high number for “X” or “Y” means that the patent application has relevant background citations. Only those patent applications with fewer than 3 X or Y documents were retained. At last, (6) the number of claims determines the scope of the invention. ‘Many claims’ means that several features can be

protected by the patent. Patents with at least 10 claims were retained. Forward citations are not considered here because most of the patents were very recent. Only those patents that strictly referred to concentration and separation techniques in their abstracts were retained.

### 3. Results and discussion

#### *A. Literature overview*

A 1-ha-scale open-pond microalgae biomass production facility with a minimum productivity of 35 ton biomass ha<sup>-1</sup> year<sup>-1</sup> would need to process 200–300m<sup>3</sup> of microalgae culture daily. This means that 99.95% of that volume is water that needs to be separated from the biomass, assuming a biomass concentration of 0.5 g L<sup>-1</sup>. Unfortunately, most of the reported harvesting techniques are only tested on a lab, bench or pilot scale (Table 1). Pilot scale data are available for centrifugation and filtration-based methods. Spiral plate rotor technology (SPT) is based on centrifugation optimized for microalgae separation by the Dutch company Evodos. Thin films of microalgal suspensions are subjected to centrifugal forces between plates. A high throughput has been reported [28], but the discharge is discontinuous with a maximum throughput of only 4 m<sup>3</sup> h<sup>-1</sup>. Decanter centrifugation is another centrifugal separation technique that consists of two concentric rotating elements that operate continuously [29]. Both centrifugal techniques achieve a high TSS, but published data for decanters has been limited to bench scale setups [30].

Filtering techniques generally consume less energy than centrifugal techniques. This is particularly true for belt filtration and microstrainers. Belt filtration consists of two belts that squeeze the liquid from the solids, resulting generally in a high TSS. Moreover, it has a high flow throughput up to 200 m<sup>3</sup> h<sup>-1</sup> for large algal species [31]. Unfortunately, the performance of filtering techniques generally depends on the microalgal species. Filtration is only sustainable for long-length microalgae or those which form large colonies, but cultures with low microalgal

cells concentration can also be harvested [7]. Microstrainers consist of a rotating drum with a belt and a backwash spray, while vacuum belts make use of a continuous belt with a suction force. A chamber filter press uses plates with filter medium that build up a cake that must be regularly removed. Tangential flow filtration (TFF) is a separation technique that uses cross-flow filtering membranes. The energy consumption for this particular technique varies strongly and depends on the operating pressure ( [32]; [33]; [34] ). However, screen clogging and membrane fouling remain important limiting factors for filtration techniques, as they increase operational costs [33]. The CAPEX for belt filter presses was generally lower than for centrifugation techniques and some authors concluded that microstrainers were more cost effective than centrifuges, i.e. disc centrifuges [35].

**Table 1: Microalgal harvesting methods overview: comparison based on strain limitation, final total suspended solids concentration (TSS), capital expenditures (CAPEX), operational expenditures (OPEX), energy demand and greenhouse gas emission (GHG)**

Harvesting method	Strain limitation	TSS (%)	CAPEX (\$ m <sup>-3</sup> )	OPEX (\$ m <sup>-3</sup> )	Energy (kWh m <sup>-3</sup> ) <sup>3)</sup>	GHG (gCO <sub>2</sub> -eq MJ <sup>-1</sup> )	Scale <sup>4</sup>	REFs
<b>Primary concentration/separation</b>								
<b>Sedimentation</b>	high	0.5–3	0.03	0.05–0.39	0.05–0.1	2.11–28	pilot	[36] [37] [38] [39] [29] [15] [40] [16] [41] [42] [43] [44] [45] [22] [46] [47]
<b>Auto/Co-flocculation/biofilms<sup>1</sup></b>	medium	1.4–5	0.03	0.06–1.5	0.02–0.2	10	pilot	[48] [36] [15] [40] [49] [50] [46] [51] [52] [53]
<b>Inorganic flocculation</b>	low	1.2–7	0.36	0.53–2.26	0.00084–2.85	1.26–36	pilot	[54] [31] [18] [39] [29] [23] [24] [55] [25] [50] [51]
<b>Organic flocculation</b>	medium	0.6–15	0.26	0.1–21.45	0.1–14.81	8.88–56	lab	[26] [56] [32] [18] [39] [57] [29] [24] [16] [49] [50] [58]
<b>Electrolytic flocculation</b>	low	3–5	0.05–6.03	0.11–1.45	0.04–9.5	47.9	bench	[54] [15] [34] [44] [45] [50] [46] [62] [26] [60] [61] [62]
<b>Magnetic flocculation</b>	medium	4.4	1.02	0.62	6.5	65	lab	[63] [41] [64] [65]
<b>Hydrocyclone</b>	high	0.4	4.32	1.87	0.3	160	bench	[39] [29] [16]
<b>Dissolved air flotation (DAF)</b>	medium	1–8	1.46	0.26–1.80	0.6–20	57.8–80	pilot	[36] [26] [66] [67] [15] [16] [42] [44] [35] [45] [50] [46] [51] [52]
<b>Electrolytic flotation</b>	medium	3–5	1.07	0.65	0.3–2	47.9	bench	[43] [44] [45] [46] [62]
<b>Suspended air flotation (SAF)<sup>2</sup></b>	medium	1.4–5	1.04	0.65	0.003–0.015	70–90	pilot	[66] [68] [43] [50] [46]
<b>Microstrainer</b>	high	1.5–3	0.05	0.02	0.02–0.5	50	pilot	[69] [16] [41] [35] [43] [52]

<b>filtering</b>								
<b>Acoustic aggregation</b>	low	7.6	2.6	0.65	16–40	47.4	lab	[8] [26] [70]
<b>Secondary concentration/separation</b>								
<b>Decanter</b>	medium	12–30	0.58– 1.75	0.39– 1.13	3.6–10.8	39–80	bench	[18] [29] [16] [22] [51] [19]
<b>Disk stack centrifugation</b>	medium	10–22	0.48– 0.55	0.2– 1.63	0.7-1.4	53– 398.48	bench	[39] [29] [15] [71] [16] [72] [28] [73] [21] [34] [20] [45] [22] [51]
<b>Spiral plate rotor (SPR)</b>	low	31.5	0.41– 0.78	0.2– 0.39	0.42–1.94	242	pilot	[74] [38] [75] [71] [28] [76] [77] [50] [62]
<b>Membrane filtration/TFF<sup>3</sup></b>	medium	2–27	1.87	0.35	0.012-10	40-45.4	bench	[78] [79] [80] [26] [66] [56] [32] [71] [41] [73] [33] [34] [52]
<b>Belt filtering</b>	high	12–50	0.29– 0.88	0.18– 0.57	0.16–0.88	20–79	pilot	[69] [81] [82] [18] [83] [29] [16] [41] [20] [46]
<b>Chamber filtering</b>	high	5–27	0.19	0.07	0.88	1.65– 241.87	no data	[66] [39] [29] [16] [21] [45] [19]
<b>Vacuum belt filtering</b>	high	9.5–18	0.42	0.18	0.1–5.9	92.5	pilot	[69] [56] [39] [29] [84] [16] [34]
<b>Vibrating screen filtering</b>	medium	1–10	0.62	0.19	0.4–3	70.5	pilot	[85] [41] [45] [42] [44] [34]

<sup>1</sup>Auto- and co-flocculation are taken together; biofilms on substrata are included in this category

<sup>2</sup>Hydrophobic layer separation included

<sup>3</sup>Micro-and ultra-filtration and Tangential Flow Filtration (TFF) are combined

<sup>4</sup>Lab: volumes <10 gallons, bench: volumes 10–1000 gallons, pilot: volumes >1000 gallons [30]

Coagulation-flocculation-sedimentation is a separation technology that has been established for decades in different industries like drinking water production or wastewater treatment. Inorganic flocculation uses inorganic chemicals as flocculants, such as metallic salts, to neutralize the negative charge of the microalgae and allow floc formation. The energy requirements are acceptable compared with other flocculation techniques, but these flocculants can contaminate the biomass and limit water recovery. Moreover, inorganic flocculants are required in higher doses than organic flocculants, but are less species dependent [57]. Bioflocculation is a concentration technique that covers spontaneous (auto-flocculation) or natural flocculation (co-flocculation). Auto-flocculation is similar to sedimentation, but only requires pH regulation ( [72]; [43]; [45]; [6] ) or stress conditions ( [86]; [87] ) that promote flocculation. Both techniques have been applied on a pilot scale [30]. The costs for both sedimentation and bioflocculation were very competitive. However, the OPEX for bioflocculation strongly varies depending on the kind of additives used for pH adjustment, the kind of flocculating organisms or the potential application of genetic engineering [88]. Sedimentation and bioflocculation have a low energy consumption and low GHG emissions ( [40]; [52] ), however, the energy consumption for the growth of micro-organisms for co-flocculation should be taken into account.

Flotation can be seen as inverted sedimentation with the additional benefits of high TSS, a small areal footprint and lower operation time. Dissolved air flotation (DAF) uses air bubbles that are released after pressurization. The air bubbles attach to microalgae and carry them to the water surface. DAF can be used on a large scale ( $>1000 \text{ m}^3 \text{ d}^{-1}$ ) and a flow throughput of  $25 \text{ m}^3 \text{ h}^{-1}$  has been reported ( [35]; [42] ). However, air compression comes with a significant increase in energy demand, which will in turn increase CAPEX and OPEX to higher levels compared to, for



example, bioflocculation followed by sedimentation. Suspended air flotation (SAF) makes use of dispersed air bubbles, eliminating the compressing step. This usually results in better techno-economics despite the lower TSS.

In any case, the biological properties of microalgae species should be taken into account as well, as they might affect the overall efficiency and general applicability of microalgal harvesting methodology. The cell size, shape, density, surface charge, robustness and overall lipid content of the specific microalgal species or strain generally affect significantly the performance of most of the harvesting technologies [89]. Sedimentation for example requires large (spherical) microalgae (>100 nm diameter) [72] or microalgal flocs with a high density [90] to increase the settling velocity. On the other hand, for flotation-based separation, the size of the cells and flocs needs to be smaller to increase buoyancy and bubble attachment [45]. Secondly, the chemical composition of the growth medium will affect harvesting efficiency (pH, nutrients, salinity, temperature, density). This is particularly true for flocculation and bioflocculation techniques [12]. A high salinity inhibits organic flocculation as well [29] but could improve electrolytic flocculation [41]. Finally, the addition of toxic compounds, like heavy metals that are used as flocculants or released from electrodes, but also some synthetic polymers, can restrict the downstream use of biomass or suppress the growth of the microalgae when the medium is recycled [70]. This demonstrates that an optimal microalgal harvesting method will depend on the targeted strain and its cultivation conditions.

This literature overview shows that auto- and co-flocculation, as well as sedimentation, overall

scored best for the economic (CAPEX and OPEX) and environmental (energy and GHG) criteria, while belt filters scored the highest on the technological criterion (TSS) (Table 1). Some authors have proposed to combine bioflocculation with gravity sedimentation for microalgal biofuel production, because it seems to be the most cost-effective harvesting method ([7] ; [89]). Another recent review [90], based on a comparative analysis of harvesting methods for the industrial production of biodiesel, suggested a combination of organic flocculation and one of the following techniques: disk stack centrifuges, cross flow filtration or decanters. So far, no harvesting technique was superior on all criteria. However, the relative importance of technological (e.g. strain sensitivity, TSS), economic (e.g. CAPEX and OPEX) and environmental (e.g. energy and GHG) criteria will determine which combination of harvesting technology is most promising. For high value products it is very likely that environmental and economical criteria are less important than the technological criteria. For low value products on the other hand, the relative importance of economical and environmental criteria might be higher.

### *B. Patent analysis*

This patent search and analysis retrieved 79 EP, PCT (WO) or US patent publications since 2000. Of those, 36 were published worldwide (WO), 36 for the United States (US) and seven for Europe (EP) (Table 2). Most of the patent applications were filed for organic flocculation (nine applications), auto-flocculation (eight applications), electrolytic and magnetic flocculation (eight applications) and membrane filtration (seven applications), but magnetic flocculation and belt filtering are the technologies which represent the highest number of granted patents. In total, 36

patents are still in force, which represents 46% of the total number of applications. From these 79 patent applications, 27 were granted (20 in the US and 7 via the EP procedure), whereof 22 (5 EP and 17 US patents) are currently active. Sixteen of the 22 granted patents fall under the scope of the quality indicators, while this is only the case for six (and one under appeal) of the other 16 applications. The remaining 43 applications were lapsed, abandoned, rejected or withdrawn.

**Table 2: General overview of patent analysis: patent applications/granted in Europe (EP), USA (US) and worldwide (WO) and the total number valid or expired**

Harvesting method	EP		US		WO	TOTAL	
	Appl.	Grant.	Appl.	Grant.		Valid	Exp.
<b>Primary concentration/separation</b>							
<b>Sedimentation</b>			2		1		3
<b>Auto-flocculation</b>	2		1	1	4	2	6
<b>Co-flocculation</b>				1	2	2	2
<b>Biofilms</b>			2		1		3
<b>Inorganic flocculation</b>			1	1	1	2	1
<b>Organic flocculation</b>	1		2	2	4	4	5
<b>Electrolytic flocculation</b>				1	7	4	4
<b>Magnetic flocculation</b>	1		3	4		5	3
<b>DAF</b>	1		1		3	2	3
<b>Electrolytic flotation</b>					1		1
<b>SAF</b>					1		1
<b>Acoustic aggregation</b>			1	2	1	3	1
<b>Secondary concentration/separation</b>							
<b>Spiral Plate Rotor</b>	2					2	
<b>Membrane filtration</b>				2	5	4	3
<b>TFF</b>			1		2		3
<b>Belt filtering</b>				4		2	2
<b>Chamber filtering</b>				1	1	2	
<b>Vibrating screen filtering</b>					1	1	
<b>Hydrocyclone</b>			1	1	1	1	2
<b>TOTAL</b>		7	16	20	36	36	43

In total, eight patent applications were filed for auto-flocculation, four for co-flocculation, three for sedimentation and four for belt filters. However, only three of the granted patents ( [93]; [94]; [95]) and one of the valid applications [96] fall under the scope of the quality indicators (Table 3).

First, the patented auto-flocculation techniques require the culturing of genetically engineered microorganisms, which is currently only allowed in closed photo-bioreactors. This technology is described in EP2294179B1 by using microalgae from the genus *Chlamydomonas*, *Dunaliella*, *Scenedesmus* or *Haematococcus* [93]. An alternative could be the use of naturally occurring organisms or info-chemicals, that cause a defense reaction by the microalgae to induce flocculation [97]. More research is required to improve the technical feasibility of this method. Secondly, co-flocculation relates to the use of other micro-organisms, like bacteria [94] or fungi [96]. US8574887B2 covers microalgae from *Chlorella*, *Scenedesmus*, *Nannochloris*, *Chlamydomonas*, *Chlorococcum*, *Euglena*, *Cryptomonas* and *Ellipsoidion* [94] and in US2012282651A1, the microalgae are selected from a very large group [96]. However, the co-production of these species also requires additional energy and costs. Thirdly, sedimentation techniques are in general not frequently patented. Low flow throughput and species dependency are the major barriers [98]. As a result, none of the three retrieved patent applications is still in force. Lastly, belt filters seem to be applicable on a large scale with a reduced energy consumption and GHG. However, only one patent [95] for belt filters, that falls within the scope of the quality indicators, is still valid. In that patent US8092691B2, species from the genus *Botryococcus*, *Chlorella*, *Euglena* and *Nannochloropsis* were used to test the filter screens [95]. From 2014 onwards, a number of patent applications have been filed for membrane filtration and electrolytic techniques. One of the claimed improvements to prevent filter clogging is the use of a hydrogel to increase the effective diameter of the algal cells [99]. However, despite an increasing number of patent applications, this analysis reveals that not a single patented technology is able to meet all of the requirements to be a dominant harvesting solution for microalgae biomass.

*C. A comparison of the literature overview versus the patent analysis*

The different economical and environmental criteria that were used to compare the harvesting technologies in the literature overview are generally not specified and described in the analyzed patents. The economical and environmental advantages are only occasionally mentioned in the patent applications, but are generally not discussed in detail. It is therefore difficult to compare the technological potential of a patented system based on the criteria defined in scientific literature. It is even more difficult to interpret the provided empirical data in a patent application to determine its applicability on a large scale.

The information provided in scientific literature is based on empirical data usually collected for a single or only a few microalgae species for comparison. This means that conclusions for the studied harvesting technology cannot always be generalized. While such studies provide new knowledge and insights, they are not always directly targeted to commercial applications. The aim of a patent application, on the contrary, is to protect and legally cover crucial information required for the commercial development of a new technology. In the case of harvesting technology, this kind of protection can be applied for a harvesting device, a separation method or novel use of a known compound. The patent granting procedure requires considerable costs and investments, and it is therefore often strategized to make claims in a broad scope, for example by covering a large number of microalgal and/or cyanobacterial species. All claims should be covered by the description for consideration in the granting process, so including a large list of

microalgal species in the patent claims could raise doubts about its enablement because all of the claimed species should have been tested for the invention.

Since patenting involves making the technology freely available for others to reproduce, small but sometimes crucial improvements may not be patented and instead kept as a company secret. To minimize this risk, small but sometimes crucial improvements are therefore often not patented and kept as a company secret [100]. The publication of a defensive patent publication can on the other hand be novelty destroying.

Future research and development should be conducted into the sequential combination of low-cost concentration and separation techniques. One of the most promising routes from a techno-economic point of view is the use of auto-flocculation techniques in for example wastewater, that serves as microalgal growth medium, in combination with filtration or centrifugation.

Furthermore, the use of CO<sub>2</sub> as pH regulator should be further examined because it can be used to release the microalgal cells from their precipitants ( [101]; [102]; [103] ). Belt filters with a low differential pressure, based on the patented technology, could be applied after auto-flocculation to increase the TSS of the microalgal biomass. Finally, the integration of subsequent processes, such as controlled cell-distruption, component extraction, catalytic conversion, etc. into a single step harvesting process might be interesting future focus areas both from a techno-economical as from a intelectual property point of view.

**Table 3. Patent analysis for microalgal harvesting technologies: EP and US patent and PCT applications (WO) with the patent numbers (publication numbers), the technology type, the number of family members, the number of IPC, the number of claims, the number of backward citations and the number of X or Y documents in the search report, the number of paid renewal fees and the maximum validity date or the current legal status.**

Priority (year)	Harvesting method	Patent Number (or publication)	Inventor(s)	Claims	Families	IPC	X/Y	Granted (year)	Renewals paid	Valid till or legal status
1999	Magnetic flocculation	EP1097905	[104]	6	4	16	0	2005	15	Lapsed
2002	TFF	US2008213868	[105]	20	4	3	2	-	-	Abandoned
2007	SPR	EP2178617	[106]	15	18	2	3	2011	9	2028
2007	Inorganic flocculation	WO2009082696	[107]	30	2	2	2	-	-	Abandoned
2007	Biofilms	WO2009037355	[108]	10	10	2	5	-	4	Withdrawn
2007	DAF	EP2167431	[109]	9	29	7	0	2015	9	2028
2007	Hydrocyclone	WO2008140307		13	6	3	1	-	-	Withdrawn
2008	Hydrocyclone	US2010031561	[110]	19	1	1	2	-	-	Abandoned
2008	Belt filtering	US8372631	[111]	9	2	2	0	2013	-	Lapsed
2008	Belt filtering	US8377687	[112]	7	2	2	0	2013	-	Lapsed
2008	Auto-flocculation (genetic)	EP2294179	[93]	15	14	4	4	2014	7	2029
2008	Co-flocculation (stress)	WO2010036334	[113]	24	2	1	4	-	-	Withdrawn
2009	SPR	EP2475461	[114]	13	2	3	6	2013	7	2030
2009	Membrane filtration	WO2010085619	[115]	22	5	2	1	-	3	Withdrawn



<b>2009</b>	TFF	WO2010120992	[116]	28	2	3	4	-	-	Withdrawn
<b>2009</b>	Auto-flocculation (genetic)	US8404473	[117]	4	1	2	18	2013	1	2029
<b>2009</b>	Hydrocyclone	US8434626	[118]	20	2	2	0	2013	1	2030
<b>2009</b>	Sedimentation	US2010314323	[119]	21	3	1	1	-	-	Abandoned
<b>2009</b>	Electrolytic flocculation	US8772004	[120]	10	2	6	1	2014	-	2030
<b>2009</b>	Auto-flocculation (pH)	WO2011040955	[101]	12	2	2	2	-	-	Withdrawn
<b>2009</b>	Sedimentation	US2010264094	[121]	20	1	1	1	-	-	Abandoned
<b>2009</b>	Co-flocculation (bacteria)	US8574887	[94]	1	3	4	0	2013	-	2030
<b>2009</b>	SAF	WO2011008784	[122]	17	2	4	2	-	-	Withdrawn
<b>2009</b>	Membrane filtration	WO2011026482	[123]	20	3	2	2	-	-	Withdrawn
<b>2009</b>	Belt filtering	US8092691	[95]	3	9	1	3	2012	1	2029
<b>2009</b>	Chamber filtration	US8518132	[124]	23	3	1	0	2013	-	2030
<b>2009</b>	Organic flocculation	US8281515	[125]	33	4	2		2012	1	Expired
<b>2009</b>	Electrolytic flocculation	WO2010136195	[126]	18	3	1	3	-	5	Withdrawn
<b>2010</b>	Biofilms	US2011217764	[127]	26	2	1	5	-	-	Abandoned
<b>2010</b>	Auto-flocculation (genetic)	EP2441828	[128]	14	4	3	1	2015	5	Lapsed
<b>2010</b>	Organic flocculation	EP2397541	[129]	14	5	6	0	2015	7	2030
<b>2010</b>	DAF	WO2012000056	[130]	16	7	2	3	-	-	Withdrawn
<b>2010</b>	Belt filtering	US9095808	[131]	22	1	1	2	2015	-	2031
<b>2010</b>	Inorganic	US8790425	[132]	9	2	2	2	2014	-	2031

	flocculation									
<b>2010</b>	Membrane filtration	WO2012085210	[133]	15	4	2	4	-	-	Withdrawn
<b>2010</b>	Acoustic aggregation	US8889388	[134]	21	1	4	3	2014	-	2031
<b>2010</b>	Magnetic flocculation	US8399239	[102]	9	2	5	0	2013	-	2031
<b>2010</b>	Magnetic flocculation	US9464268	[135]	20	3	5	3	2014	-	2034
<b>2010</b>	Magnetic flocculation	US8828705	[136]	19	1	1	3	2014	-	2031
<b>2010</b>	DAF	WO2012047680	[137]	33	5	1	4	-	-	Withdrawn
<b>2010</b>	Organic flocculation	WO2011123970	[138]	23	6	2	2	-	4	Withdrawn
<b>2010</b>	Electrolytic flocculation	WO2012054404	[139]	43	2	3	7	-	-	Withdrawn
<b>2011</b>	Organic flocculation	WO2013063605	[140]	16	2	3	6	--	-	Withdrawn
<b>2011</b>	Electrolytic flotation	WO2013010252	[141]	14	3	2	0	-	-	Withdrawn
<b>2011</b>	Magnetic flocculation	US2012238003	[142]	12	3	2	1	-	-	Abandoned
<b>2011</b>	DAF	WO2012097981	[143]	10	2	1	2	-	-	Withdrawn
<b>2011</b>	Co-flocculation (fungus)	WO2013055887	[144]	34	1	2	4	-	-	Withdrawn
<b>2011</b>	Sedimentation	WO2012150390	[145]	10	5	1	3	-	4	Withdrawn
<b>2011</b>	Inorganic flocculation	US2014273173	[146]	15	4	2	0	-	-	-
<b>2011</b>	Auto-flocculation	WO2013059754	[147]	44	2	12	7	-	-	Withdrawn

	(pH)									
<b>2011</b>	Organic flocculation	WO2013076072	[148]	26	3	5	4	-	3	Withdrawn
<b>2011</b>	Organic flocculation	US2013026106	[149]	12	1	1	3	-	-	Abandoned
<b>2011</b>	Auto-flocculation (genetic)	WO2012139086	[150]	54	5	4	3	-	-	Withdrawn
<b>2011</b>	Magnetic flocculation (genetic)	US2013210064	[151]	20	1	4	0	-	-	Abandoned
<b>2011</b>	Organic flocculation	WO2013049553	[152]	23	5	1	2	-	5	-
<b>2011</b>	Co-flocculation (fungus)	US2012282651	[96]	20	2	4	5	-	-	Appeal
<b>2011</b>	Auto-flocculation (genetic)	US2014234904	[153]	30	2	2	3	-	-	Final rejection
<b>2011</b>	Acoustic aggregation	US2013116459	[154]	29	1	2	5	-	-	Abandoned
<b>2011</b>	Acoustic aggregation	WO2013028727	[155]	20	4	1	3	-	5	-
<b>2011</b>	Electrolytic flocculation	WO2012129031	[156]	28	3	3	2	-	4	Withdrawn
<b>2012</b>	Organic flocculation	US9267105	[157]	14	4	4	0	2016	-	2032
<b>2012</b>	TFF (microfiltration)	WO2014003988	[158]	19	2	2	6	-	-	Withdrawn
<b>2012</b>	Biofilms	US2014011246	[159]	13	1	6	6	-	-	Abandoned
<b>2012</b>	Auto-flocculation (stress)	WO2014003530	[160]	15	2	2	8	-	-	Withdrawn

<b>2012</b>	Organic flocculation	US2015284673	[161]	19	2	2	6	-	-	-
<b>2012</b>	Membrane filtration (pressure filtration)	US8980618	[162]	20	1	1	0	2015	-	2033
<b>2012</b>	Membrane filtration (pressure filtration)	US9051554	[163]	20	1	2	5	2015	-	2033
<b>2012</b>	Acoustic aggregation	US8668827	[164]	5	8	2	4	2014	1	2033
<b>2012</b>	Electrolytic flocculation	WO2014074790	[165]	20	23	7	3	-	-	Withdrawn
<b>2012</b>	Chamber filtration	WO2014041063	[166]	14	3	9	3	-	4	-
<b>2012</b>	Electrolytic flocculation	WO2013116357	[167]	20	35	2	5	-	4	-
<b>2013</b>	DAF	US2015128838	[168]	20	1	5	2	-	-	-
<b>2013</b>	Magnetic flocculation	US2014248680	[169]	20	2	4	1	-	-	-
<b>2013</b>	Magnetic flocculation	US9322013	[170]	6	4	3	4	2016	-	2034
<b>2014</b>	Vibrating screen filtering	WO2016052174	[171]	13	4	3	0	-	-	-
<b>2014</b>	Electrolytic flocculation	WO2016088057	[103]	19	1	9	2	-	-	-
<b>2014</b>	Electrolytic flocculation	WO2015196241	[172]	71	2	3	2	-	-	-
<b>2014</b>	Membrane filtration (hydrogel)	WO2015121618	[99]	38	2	4	1	-	3	-

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<b>2015</b>	Membrane filtration	WO2016168871	[173]	16	2	2	-	-	-	-
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