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Non-enclosure methods for non-suspended microalgae cultivation: literature review and research needs

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ABSTRACT

Microalgae are getting more interests from industry and science communities. Applications of these small, unicellular microorganisms are countless: from fourth generation biofuels, through fish feed to pharmaceuticals. Ordinary methods of cultivation may be associated with many problems such as high costs, high energy consumption, and low product yield. It is difficult to control contaminations in open ponds while photobioreactors are mainly at laboratory scale and expensive to scale-up. Scientists are investigating various methods of microalgae cultivation and processing to overcome those problems. One of the novel approaches is the non-suspended method for microalgae culturing, where microalgae are grown on attached surfaces.

Growing microalgae on surfaces is an attractive option and showing promising results. In comparison with ordinary suspended photobioreactors, the attached systems offer higher biomass yields, easy to scale-up with better light distribution within the reactor and better control of contamination. Moreover, the consumption of water can be drastically reduced. So far, there is not enough research for this method. Limited studies have been reported on enclosure mode of this approach with algae encapsulation into matrix. It is found that this mode would be difficult to scale up due to high costs of the enclosure material and difficulty of separating microalgae from matrix. Non-enclosure mode is more promising way of non-suspended cultivation.

So far, no work has been carried out to conduct non-suspended culturing with the use of aeroterrestrial microalgae. They are species growing on the surfaces at highly humid environments. Using them in attached cultivation systems could potentially lower the water consumption to minimum. Studies have shown that the biomass of lower water content can be produced if compared to non-suspended cultivation methods. In addition, mechanization of the cultivation and harvesting processes would be less complex, as the product will not be immersed in the liquid. There would be no need for glass reactors, as lights can be placed in the spaces between surfaces. The light distribution is predicted to be the highest among all existing methods, as there would be no free floating particles absorbing and reflecting light. It will only need humid conditions, rich in CO_2 between attachment surfaces. To evaluate potential advantages for non-suspended culturing of aeroterrestrial microalgae in non-enclosure way, proper experiments need to be conducted. In this review, basic concepts of attached cultivation system are discussed, focusing on the studies of biofilm formation including factors affecting deposition and systems. The detailed description of aeroterrestrial microalgae is included to give insight into potential applications of the species into attached cultivation systems.

Contents

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1. Introduction

Microalgae are present on the planet Earth from the very beginning of its existence. The interest in these small microorganisms is drastically increasing over the last decades, given their attractive applications in pharmaceutical and many other areas, from simple fish feed to important new generation biofuels. The list also includes specialized medicines, health and beauty cosmetics, fertilizers, and many more The[1,2].

Scientists have been working to make the production of algae more commercially viable. However, there are many challenges, harvesting is one of them [3]. Processing large volume of microalgae culture is expensive and time-consuming. One possibility is to accumulate microalgae on surfaces during cultivation to allow easy collection. To date, non-enclosure microalgae cultivation has not yet received enough attention. There is no reported study on non-suspended cultivation of aeroterrestrial microalgae. Most of the aeroterrestrial microalgae researchers focus on the problems caused by microalgal biofilm formation, by analyzing mechanisms of attachment and anti-fouling methods of growth control and prevention. This review is to discuss the potentials of using aeroterrestrial microalgae in non-enclosure microalgae cultivation. Literature on the microalgae biofilm formation on artificial substrate is investigated to establish future research routes for microalgae cultivation.

2. Microalgae cultivation: an overview

2.1. Suspended vs non-suspended cultivation

The most common approach in algae cultivation is suspended method, where microalgae are growing suspended in the medium. Algae flows freely inside the container with additional mixing to ensure even distribution of cells. This method shows low concentration of algae grown. The dilution of microalgae in suspended systems is high. Around 99% of culture volume consists of water [4] and only remaining 1% is the dry algal biomass used later on. To obtain dense product, huge biomass are needed to process. Therefore, harvesting large volume of microalgae is extremely expensive process till date [5]. Supplying water to maintain microalgae production is also of high importance. It is projected that about 3800 kg of water is required to obtain 1 kg of biodiesel [6]. Therefore, a huge amount of water is needed for processing microalgal growth in suspended cultivation systems. The productivity in current suspended systems is low [7]. So far, maximum of few grams of dry biomass per liter of media can be produced during one day of suspended cultivation The[8,9]. The productivity depends on various factors such as microalgae species, reactors and culture density. In the table below there are some selected examples of biomass productivities (Table 1):

At non-suspended mode, algae are grown on surfaces. It leads to accumulation of dense algae inside the reactor. They can be enclosed in the matrix (enclosure method) or form a biofilm on the surface (non-enclosure method) [21]. With non-suspended way of cultivation, it is much easier to separate microalgae biomass from the medium when microalgae accumulate significant quantities of biomass on small area [22]. For harvesting, algae is scratched and dried in the case of non-enclosure method. For

Table 1
Biomass productivities for different kind of suspended cultivations.

Algae specie	Productivity [mg/L per day]	Type of cultivation	Reference
Chlorella sp.	3200	Closed	[10]
	4025	Open	[11]
Chlorella vulgaris	40	Closed	[12]
	136	Mixotrophic	[13]
Spirulina platensis	320	Open	[14]
	320	Mixotrophic	[15]
	2100	Closed	[14]
Botryococcus braunii	26	Closed	[16]
	155	Closed	[17]
Scenedesmus obliquus	140	Closed	[18]
	150	Closed	[17]
Haematococcus pluvialis	76	Open	[19]
	410	Closed	[20]

Table 2
Productivity comparison of suspended and non-suspended cultivation [23].

Species	Attached cultivation productivity [g/m^2 per day]	Suspended cultivation productivity [g/m^2 per day]	Reference
Scenedesmus obliquus	70.9	8.9–14	[7]
Botryococcus braunii	5.5–5.7	2.4	The[7,23]

enclosure method, a pre-step is required to extract microalgae from the matrix.

Non-suspended method can be more commercially feasible than ordinary suspended microalgae cultivation. In attached cultivation systems, microalgae are placed on vertically arranged substratum with water supplied only to keep the surfaces wet. Such a system was introduced by Liu in 2013, reaching an average productivity of 70.9 g/m^2 per day for *Scenedesmus obliquus* [7]. In the same reactor, *Botryococcus braunii* reached productivity of 5.5 g/m^2 per day [23]. The production of biomass is given in grams per squared meters in those tests. Both results were compared with ordinary suspended cultivation (Table 2):

Costs associated with water consumption are lower in non-suspended cultivation. To manufacture one ton of microalgae, around 200 metric tons of water are consumed in suspended cultivation [6] whereas in an attached cultivation method, only 17 tons of water is needed for circulation with four tons consumed for surfaces to sustain appropriate wetness level [7]. Till now, there is no attached system operating on big scale for biofuel production, nevertheless promising results are obtained from laboratory scale experiments.

2.2. Enclosure vs non-enclosure methods

The interest in enclosure method of non-suspended microalgae cultivation is growing, as microalgae are easier to control when encapsulated inside the matrix [24]. Experiments on this particular method of cells immobilization are straightforward, given the well-established techniques used for enzymes and organelles entrapment The[25,26]. However, to separate algae from the matrix is not an easy task [21]. The compounds of enclosure may have effects on microalgae species while scaling-up the process would be expensive [27].

In non-encapsulating methods of microalgae culturing, the strain is grown on artificial substrate placed inside liquid medium. Cells have a natural tendency to form biofilm in water habitat. Wild biofilms of different microalgae species can be found commonly. By creating biofilm, it is easier for them to maintain and protect themselves from biocides, predators, and medium conditions (such as pH or temperature). There are a good variety of microalgal species capable of growing on surfaces [7]. They are found on ships, inside reactor tanks or even on the building facades The[28,29]. Biofilms are rich in different species of microorganisms, such as bacteria, fungi, or microalgae [30]. Similar depositions also take place in human organisms, such as blood platelets or dental plaque [31].

Such method has not yet been applied for microalgae harvesting while the only relevant research is on macrofouling [32]. Naturally occurring biological layers have no direct benefits. There are many examples of negative influence of biofilm creation [28], such as pollution for drinking water, reduction of thermal performance for boilers, and possible toxins generated by some algae species [33]. Scientists have been researching for biofouling control and prevention, using biocides, reversal flow, ultraviolet light, and anti-fouling coatings [28]. The knowledge in the field of biofilm prevention needs to be looked at first before starting to use this method for microalgae cultivation.

2.3. Aquatic vs aeroterrestrial microalgae

Microalgae are unicellular microorganisms, widely appearing in aquatic and terrestrial environment. They play a very important role in the ecosystems on the planet Earth. There are a large number of microalgae species with 30,000 species discovered and an estimate of possible 70,000 species [34]. They can be divided according to their taxonomic group or living environment.

Aquatic microalgae are a type of algae naturally occurring inside water reservoirs. All microelements needed by aquatic microalgae sourced from the water. The examples of those strains are *Chlorella vulgaris*, *Scenedesmus obliquus*, *Pyrocystis lunula*, or *Nannochloropsis oculata*.

Aeroterrestrial microalgae are species growing in biofilms, colonizing both natural and artificial surfaces. They can be found on roof tiles, statues, building facilities, damp rooms, rocks, trees, soil, and many more non-aquatic environments of high humidity The[35,36,37]. They may be found growing together with bacteria, fungi, protozoa, and cyanobacteria The[36,38]. Biofilm thickness can reach up to 0.1 millimeters. Example strains are *Klebsormidium* sp., *Stichococcus* sp., *Coccomyxa* sp., and *Apatococcus* sp. The [29,35,39,40]. Most of the terrestrial algae can be found in green algae groups, such as *Trebouxiophyceae* or *Chlorophyceae* [41]. However, aeroterrestrial microalgae diversity is not well understood [42]. Relevant research mostly focuses on their negative impacts on building facilities. Biofilms causes decolorization and faster weathering of deposited surfaces [36] due to microbial actions triggering breakdown of those materials. Their contribution to the surface weathering is significant, especially that their growth is faster than the growth of higher plants. Species as *Gloeothece* sp., *Chlorella* sp., *Schizotrix* sp., or *Chroococcus montanus* are good examples of terrestrial microalgae that degrade surfaces The[43,44]. Aquatic microalgae, such as terrestrial ones, are also responsible for surface degradation. They form an unwanted biofilm on ships and industrial tanks. Their biofilms can be found on any artificial surface that is immersed in natural water reservoir for a longer period of time [28].

Aeroterrestrial microalgae have a unique ability to survive desiccation for a long period [45]. They can even survive in a drought when the reproduction is stopped. In comparison with liquid cultures, aeroterrestrial microalgae are flexible and shrink during dry periods (even to around 60%) [46]. Aquatic microalgae such as *Nannochloropsis* sp. and *Scenedesmus dimorphus* are not withstanding desiccation well. After drying their growth is significantly limited [47]. In contrast, aeroterrestrial microalgae are easily revived after preservation by drying. It takes only few minutes for *Stichococcus* sp. and *Chlorella luteoviridis* to recover photosynthesis after moisturizing [29]. They are highly resistant to hostile environmental conditions as a result, variations in salinity, temperature, and UV variation do not affect them as much as on marine algae [38]. Aeroterrestrial microalgae have a high survival rate at extremely low temperature. Most of 27 aeroterrestrial species tested by Lukesova in 2008 survived cryopreservation, with survival rates above 50% in most cases [48]. There were even species exhibiting 100% of survival rate: all cells of *Cylindrocystis brebissoni* and *Chlorella fusca* species survived conservation in extremely low temperature (almost 200 °C).

3. Non-enclosure methods

3.1. Biofilm formation

Biofilms occurring in natural environment are in general created by bacteria, larvae, fungus, protozoa and microalgae The [30,49]. They can be found even in extreme and unfriendly environments, such as nuclear power plants or hydrothermal vents [50]. Formation of microalgae layer is a complex process [28] while the adhesion mechanism is not fully understood The [51,52]. It is believed that the hydrophobic reactions are driving forces for biofilm formation of hard substrates [31].

The first and probably the most important step is the creation of conditioning film The[28,53]. It is a base layer on the surface for microorganisms to grow. There are no clear evidences that the

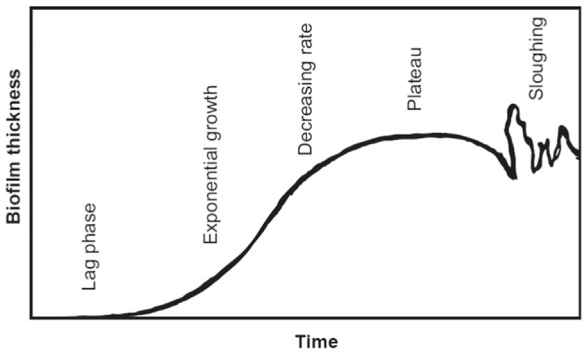

Fig. 1. Growth of biofilm in time [28].

conditioning film is required to create biofilm [54], however its formation is essential in promoting cells deposition The[55,56].

Conditioning film formation takes place straight after the surface immersed into the medium, creating the layer consisting of ions and organic molecules [53]. Once conditioning film is formed, microorganisms start their attachment. In the case of saltwater, it takes a few hours for microalgae to attach to the surface [57].

Further growth of the biofilm involves reproduction of microorganisms by division, rather than absorbing free floating particles from the surrounding medium [28]. Before reaching the exponential growth phase, microalgae undergo lag phase (time needed to start reproduction) [58]. At first few days of mixed biofilm formation, microalgae are the microorganisms that dominate the biofilm composition [59], then diatoms start to take over and eventually cyanobacteria become dominant [30].

Growth curve of microalgae in biofilm is similar to that of aquatic algae (Fig. 1). The lag phase is followed by exponential growth, then the rapid development of biofilm stops and reaches the maximum biofilm thickness, at the end the mature biofilm undergoes sloughing.

3.2. Extracellular polymeric substances (EPS)

During biofilm formation, cells produce EPS [60] to create a matrix bonding together the whole biofilm The[28,61]. This creates the environment for growth and reproduction of microorganisms and enables easy attachment of external particles [62]. EPS consists of various groups that function as metal-binding sites. Examples could be negatively charged carboxyl or phosphate group or polysaccharides, proteins, nucleic acids, lipids, phospholipids, and humic substances [63].

EPS play an important role in nutrients exchange. They are also responsible for cohesion (binding cells together) and adhesion (binding cells and substratum) [64]. EPS not only act as a nutrient sink, but also protect the whole structure of the biofilm from grazing The[65,66] and action of harmful biocides [67].

Aeroterrestrial microalgae also produce EPS. In addition to the functions above, EPS protect algae from desiccation, retaining water inside algal cells [38] to enable a longer survival during drought. It helps in survival of terrestrial species such as *Chlorella trebouxioides*, *Chlorella luteoviridis*, or *Stichococcus bacillaris* The[38,44].

3.3. Deposition factors

Various factors contribute to the growth of biofilm with nutrients and lights generally regarded as the most important for microalgae The[28,61]. For aeroterrestrial microalgae, the key factors for growth also include water availability The[29,35,42,68] and chemical/physical properties of the surfaces [53]. The type of surface is very important, as some attachment surfaces can store the water and the storage ability increases with porous materials [68]. Aeroterrestrial algae prefer rough and porous materials [44] and smaller temperature amplitudes. It is because smaller variation in temperature and presence of cracks keep the surface wet and prevents it from water evaporation The[43,69]. Nutrients concentration is also significant in the case of aeroterrestrial microalgae, however it does not have as much influence on the biofilm composition as other factors mentioned before The[70,71].

Other factors affecting the adhesive strength, amount of biomass formed and its composition could be: disturbance [72], surface roughness The[3,21,73], pH [73], surface rugosity [74], irradiance [75], fluid velocity [76], and concentration of free-floating cells in the medium The[76,77]. More details about their influences on biofilms will be discussed in the following chapters.

To investigate the influence of different factors on biofilm formation, it is important to establish parameters measuring its growth including biofilm thickness, cell counts, or dry mass of formed biofilm [78]. A typical apparatus used for monitoring the biofilm is the “Robbins device” The[28,53]. In Robbins device, test plates are placed inside aluminum block. The liquid is passing through flow channel and the biofilm is formed on test plates. It is possible to remove those plates later and study the accumulation of biomass as well as the influence of liquid velocity. The other techniques for cell biomass or biofilm activity measurement are scanning electron microscopy (SEM), transmission electron microscopy (TEM), scanning controlled laser microscopy, adenosine triphosphate (ATP), total organic carbon (TOC) measurement, light microscopy, and Confocal Scanning Laser MicroscopyThe [28,53,78,79].

All the factors mentioned above have an influence on the development of microalgal biofilm and its composition, however the extent to which they are affected mostly depends on the strain to be attached [21].

3.3.1. Light intensity

The availability of light determines the presence of microalgae in naturally occurring biofilms [28]. The intensity can increase or decrease their adhesion. The attachment is weaker with limited light and generally microalgae growth increases with light intensity [61]. Once the growth reaches its limit, cells undergo photoinhibition and the growth declines.

Light intensity works differently for aeroterrestrial microalgae. The study on *Stichococcus* and *Chlorella luteovirdis* species showed [80] that aeroterrestrial microalgae exhibit high tolerance to UVA and UVB radiation. It is due to the presence of mycosporine-like amino acids (MMA), absent in Ulvophyceae or Chlorophyceae group. Aquatic alga, *Desmodesmus subspicatus*, was affected by too high irradiation by slowing its pace of growth. Some of aquatic microalgae species can stop their growth at all in the presence of UVA and UVB radiation (Table 3) [80]. Great tolerance of aeroterrestrial microalgae to variations of light intensity is very advantageous, as the photoinhibition of cells is a main problem while culturing algae in biofilms [81].

3.3.2. Nutrient concentration

Nutrients are essential for the development of microalgae film. The amount of nutrients should be maintained at a proper level. Above that level, the attachment of cells stops increasing The [55,82]. In mature biofilms, cells closest to substratum surface have limited access to nutrients [28]. It results in their death and sloughing of whole biofilm. The needs for appropriate nitrogen, phosphorous and other elements level strongly depend on microalgae strain. Some species require extra amount of silica [83]. Addition of glucose to biofilms can enhance the accumulation,

Table 3
The effect of PAR, UVA and UVB radiation on selected algal species [80].

Conditions						
Photosynthetically active radiation (PAR)					**50 PPF**	
Ultraviolet radiation (UV)					**8 W/m² UVA 0.4 W/m² UVB**	
Specie	Type	MMA	Growth in PAR+ UVA	Growth in PAR+UVB	Recovery in PAR+UVA	Recovery in PAR+UVA/B
Stichococcus sp.	Aeroterrestrial	Yes	No change	No change	Full	Full
Chlorella luteoviridis	Aeroterrestrial	Yes	No change	No change	Full	Full
Myrmecia incise	Aeroterrestrial	Yes	30% decline	43% decline	Full	Full
Desmodesmus subspicatus	Aquatic	No	33% decline	Inhibition	Full	80%

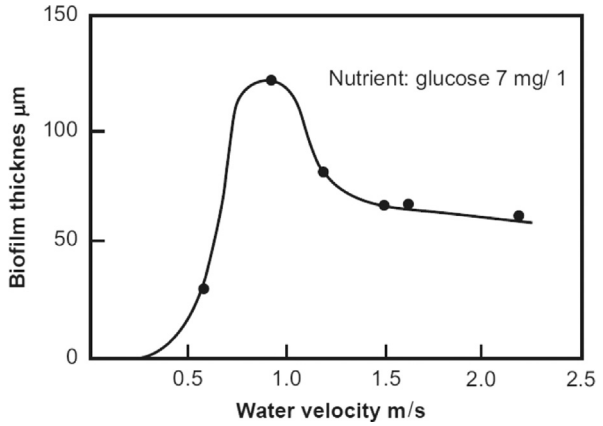

Fig. 2. Dependence of biofilm thickness on water velocity [87].

however the structure formed is loose [76]. Aeroterrestrial algae are able to withstand extreme or harsh conditions, although nutrient rich surfaces are more favorable [36]. The composition of nutrients needed by aeroterrestrial microalgae is the same as in the case of aquatic microalgae, unique for each species.

3.3.3. pH

pH of the cultivation medium affects microalgal growth and biofilm establishment [84]. The structure is influenced by pH even more than by nutrients [39]. It can also happen that the pH within microalgal layer is different from the surrounding medium [76] when microorganisms create a whole new environment separated from the surroundings during biofilming. What is the most favorable pH in the case of microalgae biofilming? The best attachment of *Nitzschia amphibian* to titanium and glass was obtained approximately at pH neutral environment [73]. This is within the acceptable range given most algae species grow well at pH level from 7 to 9 [85]. The exceptions are green algae found in soils, which prefer acidic conditions [86].

3.3.4. Flow of medium

Movements of surrounding medium influence the biofilm thickness [28] and could be one of the most important factors affecting adhesive strength [76]. Laminar flow, occurring at low velocities, generates thick laminar sub-layer, which enables material to accumulate in dispersed manner [28] and make it easy to remove cells. When the fluid velocity increases, the mass transfer between particles floating in the medium and biofilm increases as well. In the range of 0.6 to 1.6 m/s of fluid velocity, the strength of attachment is improved for *Pseudomonas fluorescens*. However, the removal of cells from the existing biofilm is also enhanced. An optimal flow velocity could be found to achieve the maximum growth of microorganism's layer (Fig. 2) [87].

Aeroterrestrial microalgae do not grow within the fluid and the movement of fluid hardly affects them, rather than the presence of water in the form such as rain, highly humid air, fog, or snow [29].

3.3.5. Strain selection

Strain selection is probably the most important for the production of microalgae in non-suspended mode. Microalgae species have different characteristics and behavior. To give insight into differences between microalgal strains, examples are presented below:

- Preferences in way of cultivation: It is evident that some strains may prefer to grow on surfaces while others grow more favorably within the medium as shown by the comparison between Bristles Photobioreactor (PBB) and Bubble Column Photobioreactor (PBC) [88]. It is found that *Amphora* sp., *Navicula* sp., and *Nitzschia ovalis* strains preferred attachment on surfaces with the best results in terms of concentration and biomass yield [88] in PBB (non-suspended cultivation). In contrast, *Nitzschia* sp. and *Cylindrotheca closterium* grew better inside ordinary PBC (suspended cultivation).
- Preferences in medium properties: It is reported that *Chlorella vulgaris* forms thicker biofilm on unsterilized medium and no such observation was found in the case of *Scenedesmus obliquus.*
- Different predispositions to create biofilms: It was found that not all algal species are capable of producing extracellular polymeric substances (EPS) which affects the quality of biofilms created [89]. An example of such microalgae is *Chlorella vulgaris*, unable to produce EPS by itself.
- Different influence on attachment surface: For non-suspended culturing, microalgae species may have direct influences on the attachment surfaces. It is found that some microalgae acts as precursors in microbiologically induced corrosion [90] due to the change in pH value and oxygen release during biofilm formation. However, not all algal species induce the biocorrosion of the substrates. It is reported that *Porphyridium purpureum* does not contribute to steel corrosion [91].
- Strain-specific approaches in attachment improvement: It is possible to stimulate some algal species to accumulate on surfaces. $CaCl_2$ was added to improve *Chlorella sorokiniana* to form aggregates [92]. Growing *Chlorella vulgaris* in non-sterile water led to more cells attached to the substratum [89].

3.3.6. Substrate properties

Characteristics of the surfaces are critical for layer formation. According to studies, following factors need to be considered, when designing the process of particle deposition:

- Roughness and texture of surface: They play important role in particles deposition, microalgae grow better on rough surfaces The[3,21,73,74,93,94]. The study of the red algae showed that *Halosaccion glandiforme*, attached to substrata with features had about 35 times larger density in comparison with density obtained on smooth surface [3]. Also the proper size of substratum dimples can elevate attachment. When dimples are slightly larger than the size of the cells to be deposited, the attachment is higher The[21,95].
- Hydrophobicity: Biofilms are created on hard substrates generally due to hydrophobic reactions [31]. To encourage microorganisms' attachment, it is preferable to have hydrophobic surfaces in particular for saltwater [96].
- Presence of protective layers on surface: Bacteria and diatoms have strong tendency to colonize surfaces, so they can be met even on specially designed antifouling coatings [97]. However, it needs to be kept in mind that microorganisms are less likely to colonize on substratum covered with hydrophilic coating [98]. It is also important to take into account the influence of attachment surface sterilization, as this process changes the properties of the surface [91].
- Costs of surface production: To make non-suspended algae cultivation feasible, the substrate materials need to be cheap and environmental friendly [5]. Surface texturing, desirable in particle deposition enhancement, should be an efficient and not cost consuming process The[5,73].

What are the materials suitable for particle deposition of microalgae? In his work on microalgae attachment, Cui tested a great variety of substrates: teflon, polycarbonate, polypropylene, nylon 6/6, glass microscope slides, and stainless steel 304 [21]. Microalgae tend to accumulate most on the material with the lowest surface free energy, nylon (34.6 ergs/cm^2) (Fig. 3). Surface free energy has bigger impact on particle deposition than surface roughness, as stainless steel possessing the roughest surface (124 nm) from all tested materials was not attaching the highest amount of cells [21].

In another study, Sekar conducted experiments with perspex, titanium, stainless steel 316-L, glass, copper, aluminum brass, and admiralty brass. His results showed that the highest attachment took place on stainless steel and titanium (Fig. 4). Remaining materials exhibited weaker promotion of microbial adherence [73].

During studies on rotating algal biofilm system, Gross showed that the most effective material for growing microalgal biofilms for biofuel production is cotton [99]. It was better than other tested materials, such as microfiber, fiberglass, nylon, or vermiculite. The same conclusion was made by Christenson. According to his research, cotton cord was more effective than nylon, polypropylene, acrylic, or jute [100].

Other studies on microorganisms attachment involved polydimethylsiloxane, polyimide, polycarbonate plates, silicon, alkane thiolates, plexiglas, and poly-dimethyl silozane elastomer (PDMSe) The[32,74,89,101–107]. All materials above were tested regarding the mechanism of attachment, contact angle data or antifouling properties rather than information which material is the best for growing microalgae in biofilms.

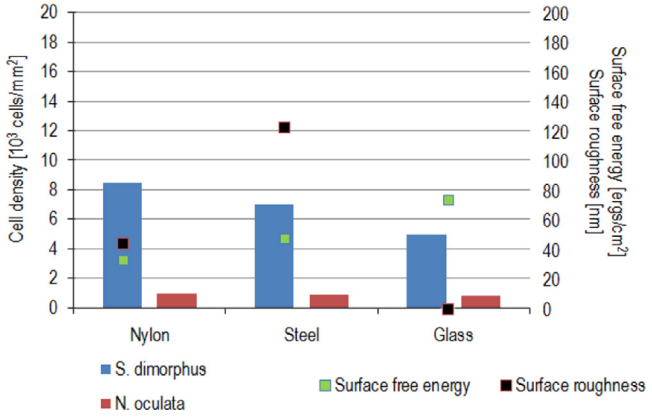

Fig. 3. Growth of *Scenedesmus dimorphus* and *Nannochloropsis oculata* on nylon, steel and glass. Surface free energies and surface roughness are given. Data based on work conducted by Cui in 2013 [21].

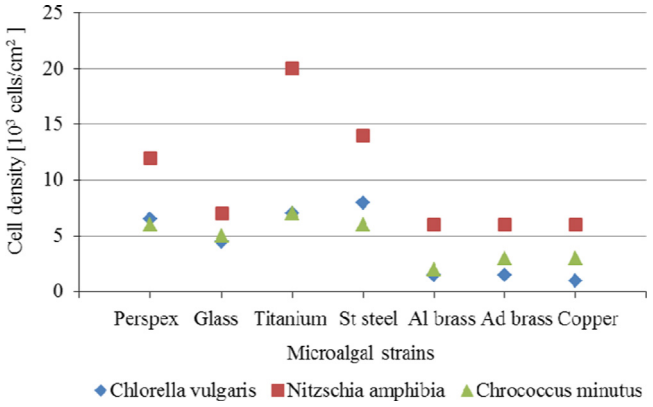

Fig. 4. Growth of *Chlorella vulgaris*, *Nitzschia amphibia*, and *Chrococcus minutus* on different materials. Data taken from study conducted by Sekar in 2004 [73].

Table 4
Biofilm reactors to treat wastewater with removal efficiencies.

Reactor	To clean	Reactor/culture volume	Removal efficiency [%]						
			TN	TP	COD	TSS	TOC	S^{2-}	NH_4-N
Parallel plate microalgae biofilm reactor [112]	domestic wastewater	3L+6L	67	96	74	82	–	–	–
Vertical submerged biofilm reactor [109]	synthetic wastewater	18L	82.7	–	–	–	–	98.2	–
Enclosed biofilm tubular reactor [111]	swine slurry	7.5L+0.5L	94–100	70–90	–	–	61	–	94
Moving bed biofilm reactor [113]	raw water from Taihu Lake	45 L	–	–	–	–	–	–	63.1
Attached algal culture system [115]	dairy from wastewater	0.05 L+0.15 L	79	90	–	–	–	–	–

TN- total nitrogen
TP- total phosphorous
COD- chemical oxygen demand
TSS- total suspended solids
TOC- total organic carbon
S^{2-}- sulfide
NH_4-N- ammonium

4. Application of attached systems

4.1. Microalgal biofilms in wastewater treatment

There is no much research conducted on microalgal biofilms devoted to biofuels production [108]. Most of the studies are on application of algae biofilms to treat wastewater. Those methods of cleaning wastes have certain advantages. They operate at low temperature and pressure, and there is no requirement for catalyst [109]. In addition, Biofilm processes are not only environmentally friendly treatments, but also effective in terms of procedure expenses The[110,111]. Examples of reactors to treat wastewater with the use of microalgal biofilms are as follows:

- PPMB Reactor: Parallel Plate Microalgae Biofilm Reactor (PPMB) was designed to immobilize nutrients from chemically treated household wastewater [112]. Nitrogen and phosphorous were removed by algal biofilm. The overall removal efficiencies of the system were satisfactory, 67% removal of total nitrogen and 96% removal of total phosphorous. The amount of total chemical oxygen demand and suspended solids was also reduced (by 74% and 82%, respectively).
- VSB Reactor: Vertical Submerged Biofilm Reactor was used to remove nitrogen and sulfide from synthetic wastewater [109]. Fixed-bed reactor made of polyvinyl chloride was able to remove 82.7% of total nitrogen and 98.2% of sulfide at third stage of the process.
- EBT Reactor: In this experiment, *Chlorella sorokiniana* was growing on walls of Enclosed Biofilm Tubular Reactor. In reactor made from transparent polyvinyl chloride, microalgae biofilm was used to treat piggery wastewater [111]. Algae biofilm was capable of removing carbon, ammonium, and phosphate.
- MBB Reactor: Apart from domestic wastewater, microalgae biofilms can be also used in treatment of raw water polluted by industrial activities. In 2013, Zhang tested Moving-Bed Biofilm Reactor (MBBR) for nitrogen removal, obtaining promising results [113].
- PRBC Reactor: Microalgae biofilms are helpful in removing nitrogen and phosphorous, but they can be also used in lowering the concentrations of heavy metals, such as copper, nickel or manganese. Photo-Rotating Biological Contractor was used to attach algae and microbes, which were efficiently removing heavy metals from mining wastewater [114]. From 20 to 50% of various heavy metals were taken away by the biofilm deposited on polyvinyl chloride disks partially immersed in acid mine drainage. Algae-microbial biofilm was able to withdrawn metals such as zinc, antimony, selenium, cobalt, aluminum and, as mentioned earlier, copper, nickel, and manganese. The summary of those studies is given in the table (Table 4).

4.2. Microalgae biofilms in biofuels production

There are only few studies on microalgal biofilm devoted to biofuel production [102]:

- Effect of nutrient starvation on lipid content: As it was proved by other researchers, this way of stressing algae is resulting in increase of lipid content and is so far the most common approach to increase fatty acids content in suspended cultivation The[102,116]. Unfortunately, the same effect on microalgae growing in biofilms was not observed. Lipid content was not elevated by nutrient starvation for *Scenedesmus obliquus* and *Nitzschia palea* [108]. After three days of starvation, the concentration of lipids did not changed and stayed on the level of 15% and 6% for *N. palea* and *S. obliquus*, respectively. When cultured at suspended mode, the same algal strains reached lipid level of 30% (*N. palea*) and 17% (*S. obliquus*) after three days of starvation. Nutrient starvation was not increasing the lipid content of microalgae, when grown in biofilms.
- Rotating Algal Biofilm Cultivation System: Gross constructed a Rotating Algal Biofilm cultivation system, in which he tested 16 materials as attachment surfaces [99]. The reactor was partially immersed in liquid medium. Rotations of reactor allowed the biomass that grows on substratum to alternatively enter liquid rich in nutrients and atmosphere with higher concentration of carbon dioxide. Similar approach was presented year earlier by Christenson (Fig. 5). His reactor achieved much better results regarding the biomass and fatty acid methyl esters (FAME) productivity in comparison with reactors in which microalgae were cultured at suspended mode [100]. Both studies showed that the best material for microalgae attachment is cotton. It is cheap, easy to acquire and as an attachment surface allows microalgae to achieve the highest biomass yields The[99,100].
- Attached Algal Culture System: In 2009, Johnson constructed system to grow *Chlorella* species intended for biofuel production with simultaneous nitrogen and phosphorous removal. Materials tested as a substrate were polystyrene foam, cardboard, polyethylene landscape fiber, loofah sponge, polyurethane foam, and nylon sponge. Among all these materials, the best in terms of biomass and total fatty acids production was polystyrene foam [115]. It was also easy to remove an algal biomass from this material and re-use polystyrene after the process (Fig. 6). The material to attach cells was placed at the bottom of moving tank. Without water movement algae tended

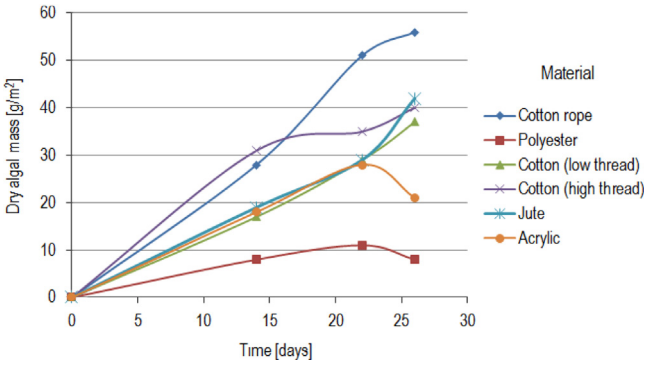

Fig. 5. Growth of mixed microalgae culture on different materials. Data taken from study conducted by Christenson in 2012 [100].

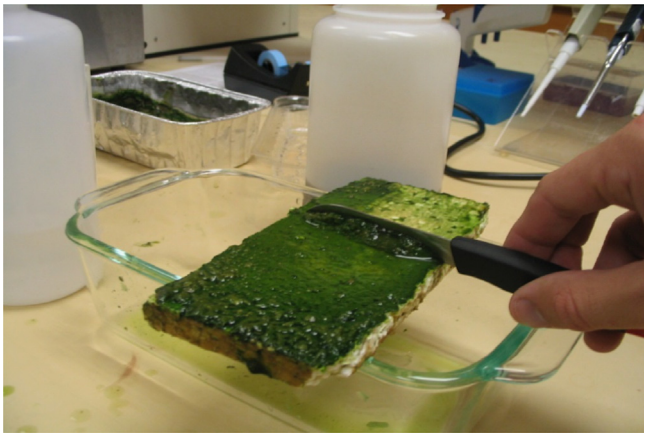

Fig. 6. Removing microalgae biofilm from attached cultivation system [115].

to accumulate at the bottom and created sediment rather than attach to the substratum. System was able to produce 3.2 g/m^2/day of microalgae. The lipid content was around 9%, which is much higher in comparison with maximal 5% of terrestrial crops. Dairy from wastewater was applied as a medium. Removal of total nitrogen and total phosphorous reached level of 79% and 90%, respectively [115].

4.3. Other experiments

Apart from systems to treat wastewater and produce microalgae for biofuels, there exist researches on other applications of microalgal biofilms:

- Light/Electricity Conversion System: Biofilm can be applied to obtain energy. Biofilm-Based Light/Electricity Conversion System was developed to exchange light irradiation energy into electric current [117]. Green algae were used in the experiment, however they were working only in the presence of heterotrophic bacteria. When the light reaches the reactor, extracellular electron transfer takes place. Electric current is generated.
- BOD removal: It is also possible to remove Biological Oxygen Demand (BOD) by application of microalgal biofilm inside Flat Plate Photobioreactor (FPP) and Tubular Packed Photobioreactor (TPP) [81]. Microalgal-bacterial biofilm is created either on beds carriers or strictly on reactor's walls. From both approaches, the second one is the most convenient, as it is not possible to achieve stability when biofilm is attached to beds carriers. In both biofilm reactors (FPP and TPP), removal rates of 92 and 108 mg BOD/L/h were achieved, in comparison with 77 mg BOD/L/h achieved in ordinary suspended reactor. It means that it is possible to conduct efficient BOD removal process with the use of microalgae and bacteria biofilm. However, the process still has certain drawbacks. Photoinhibition lowers the operation performance and the biomass accumulation during growth phase could result in reactor blockage [81].

5. Aeroterrestrial microalgae: research needs

There is a strong need for a research on aeroterrestrial microalgae which focus on their applications in industry. Present studies, as mentioned earlier, are directed towards biofouling prevention. The potential of aeroterrestrial microalgae in biofuel production, pharmaceutics industry and other areas is not known. It is essential to investigate their properties and applications, so they can be compared with aquatic species.

Growing aeroterrestrial microalgae by non-suspended cultivation is the most intuitive step in research, than should be taken as soon as possible. This review shows that non-suspended cultivation is a promising approach in microalgae culturing, and from the natural tendency of aeroterrestrial microalgae to create biofilm it can be assumed that this kind of cultivation will be the most appropriate.

6. Conclusions

Growing microalgae in attached non-suspended systems is a novel concept. Biomass yield is comparable or higher than the same species grown at suspended mode of culturing. Consumption of water is much lower, which contributes to decreasing the costs of production. Distribution of light is improved, as it is not limited by the density of culture. Most of the cells are attached to substrate; only small part is free-floating within the medium and absorbing the light. The most significant advantage of attached systems is no need for harvesting step. It was proven that the water content of microalgae scrapped from substratum is comparable to this of biomass after centrifugation. Avoiding this expensive and time-consuming step makes the algae production more feasible.

Application of aeroterrestrial microalgae is potentially decreasing the costs of production even more. The usage of water will be decreased to minimum, as algae will be grown in humid atmosphere, not in the medium. The light distribution is also expected to be enhanced, as no medium or floating cells would be absorbing the light. In addition, maintenance, mechanization, and scaling-up of the whole system should be easier, as huge volume of water does not obstruct operations inside the reactor.

Future work includes design of special reactor, in which humid atmosphere rich in CO_2 will be created and maintained. In addition, selection of proper substrate material is important, as there are no studies on the most effective substratum to grow aeroterrestrial microalgae. To find out whether this kind of microalgae could be feasible competitor to ordinary microalgal cultivation, those investigations should be carried out in the nearest future.

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