Current Topics in Biotechnology
Vol. 7, 2012

Review

Applications of the airlift loop effect in the cultivation of microalgae

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ABSTRACT

An airlift loop bioreactor (ALB) with microbubble dosing was used to grow microalgae on high CO_2 content steel plant exhaust gas, generated from the combustion of offgases from steel processing. The gas analysis of CO_2 uptake in the 2200 litre bioreactor showed a specific uptake rate of 0.1 g l^{-1} h^{-1}, an average 14% of the CO_2 available in the exhaust gas with a 23% composition of CO_2. This uptake led to a steady production of chlorophyll, biomass and total lipid content in the bioreactor, with a best doubling time of 1.8 days. The gas analysis also showed anti-correlation of CO_2 uptake and O_2 production, which along with the apparent stripping of the O_2 to the equilibrium level by the microbubbles, strongly suggests that the bioreactor is not mass-transfer limited, nor O_2 inhibited. Subsequently, an array of 3 litre laboratory bench ALBs have been developed for screening purposes, with the notion that conventional shake flask incubation for screening is oxygen inhibited. The small ALBs achieve accelerating exponential growth, resulting in the desired levels of algae density an order of magnitude faster than the undosed control. Large-scale screening time in industrial laboratories can thus be decreased significantly while using environmental conditions appropriate for full scale production, including stack gas as part of the medium. Finally, microbubble gas exchange with an airlift loop effect is not limited to photobioreactors. The circulation and mixing benefits can be replicated by engineering algal ponds, as the baffles and diffusers needed to direct the airlift loop effect are inexpensive.

KEYWORDS: algal growth, microbubbles, bioreactor, ponds

INTRODUCTION

The biotechnology of microalgae can be traced back some 60 years to the late 1940s and early 1950s, when work in the US, Japan and Germany examined the mass cultivation of microalgae for food [1]. Mass cultivation was established in Japan with growth of *Chlorella* to produce human food and animal feed products [2]. The *Chlorella* industry in Japan then developed along the lines of growing the algae either mixotrophically (in the light, but including e.g. acetate in addition to CO_2) or utilising full heterotrophic growth in the absence of light [3]. Therefore, early in the development of algal mass culture, a number of very different modes of cultivation had been established.

Elsewhere in the world, a different route was taken to commercialise microalgae, by utilising extremophilic algae that often grew naturally as virtual monocultures. The two microalgae to emerge

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in the 1980s and 1990s as commercially productive strains were halophilic strains of *Dunaliella* for β-carotene synthesis [4] and alkaliphilic strains of *Arthrospira* (formerly called *Spirulina*) - a cyanobacterium for food, feed and fine chemicals [5]. For both *Dunaliella* and *Arthrospira*, commercial production is outdoors in open raceway ponds, this cultivation system is largely restricted to growth of extremophile algae [6].

Despite the success of the raceway ponds, research has continued into closed systems (photobioreactors, PBRs) which can be used to grow a much wider range of microalgae. The vast majority of commercial scale PBRs are situated outdoors to take advantage of sunlight and consist of stirred tank and airlift bioreactors [6], tubular bioreactors (both horizontal and vertical) [6, 7] and helical, flat plate and α-shaped bioreactors [8, 9]. It should be noted that all configurations of PBRs require an airlift component to allow for efficient gas exchange.

In the present review, we examine ways that PBRs can be made more efficient by utilizing microbubbles that very significantly improve the exchange of CO_2 and O_2 in algal cultures grown in airlift loop bioreactors (ALBs). The utility of microbubbles is demonstrated in a 2200 litre ALB situated outdoors (dosed with flue gas) and in a series of laboratory bench 3 litre ALBs ideal for screening purposes. The use of microbubble gas exchange is also discussed in the context of engineered algal raceway ponds.

Fluidic oscillator (FO) driven airlift loop biorectors (ALBs) for microalgal culture

Insufficient gas-liquid mass transfer, undesirable mixing properties and O_2 inhibition are always the major concerns for design and scale-up of PBRs, which have given rise to different types of airlift bioreactors. FO driven ALB is a new design among airlift bioreactors, combining airlift loop effects and microbubble dosing benefits to achieve a better gas-liquid mass transfer and mixing/circulation [10]. For such a brand new microalgae culture system, only a few studies have been carried out so far on its parameters and performance, with respect to both laboratory trials and pilot plant trials.

Pilot study with FO driven ALB for growth of microalgae and CO_2 fixation

The microalga *Dunaliella salina* CCAP 19/30 was cultivated in a pilot scale ALB driven by one fluidic oscillator (Figure 1), using steel plant exhaust gas which has a high concentration of CO_2 (23%) as the sole carbon source [10]. The feasibility of directly using flue gas for algal cultivation was doubted by many researchers as the high CO_2 content and the presence of NO_x/SO_x might poison the culture [11, 12, 13]. However, the high CO_2 content could be favourable to microalgal metabolism while the high O_2 level accumulated due to their growth may turn out to be the limiting factor. The culture should not be inhibited but improved by high concentration of CO_2, if O_2 could be efficiently removed by microbubbles. According to the gas analysis (Figure 2), it was found that 14% of the CO_2 available in the flue gas with a 23% composition of CO_2 was captured by FO driven ALB, equivalent to 0.1 g L^{-1} h^{-1} of specific uptake rate. Meanwhile, a constant stripping of the O_2 to the equilibrium level was also detected during microbubble dosing. The results strongly supported the view that FO driven ALB culture is neither CO_2 limited nor O_2 inhibited, and good growth of biomass was achieved [10].

Laboratory ALB cultures of *Dunaliella salina*

Ying *et al.* [14] set up a series of microalgal laboratory cultures to test the efficiency of FO driven ALB culture compared with basic airlift bioreactor and traditional shake flask culture. An array of 3 litre ALBs have been developed based on the same design (Figure 3). Six ALBs were connected to a fluidic oscillator and aerated with microbubbles, while another six were dosed with conventional bubbles, running as basic airlift reactors. The thirteenth bioreactor was run under the same conditions, however without aeration, representing traditional shake flask culture. Cultures were incubated under sufficient illumination, and daily dosed with 5% CO_2 for 30 minutes, without any additional nutrients, buffer solutions or pH/temperature control devices applied. The results strongly suggested FO driven ALB cultures were cost competitive and efficient. First, it was found that a suitable pH level (6.5-9) for *D. salina* culture was achieved by 30 min day^{-1} of 5% CO_2 dosing;

Figure 1. The setup of ALB for pilot trials.

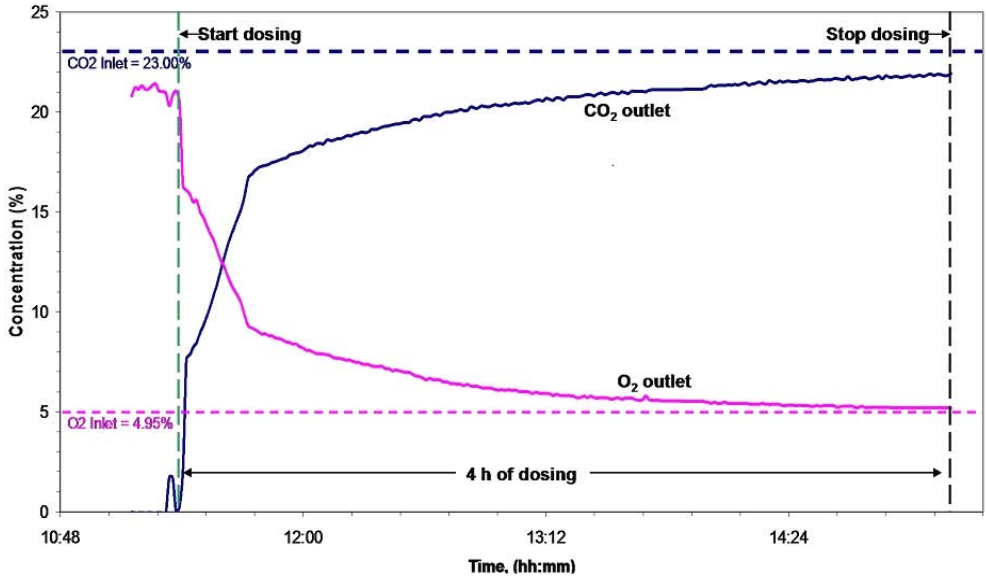

Figure 2. An example of gas analysis from the ALB.

this is shown in Figure 4. It indicates a potentially large capital cost saving on buffer solution (e.g. HEPES) which is usually expensive especially for scale-up culture. Second, microalgae in ALBs grew an order of magnitude faster than in control experiment (Figure 5), achieving the same levels of cell density (as measured by chlorophyll content) in one/third of the time taken by shake flask culture. Since industrial phycology laboratories conduct several thousands of screens per day,

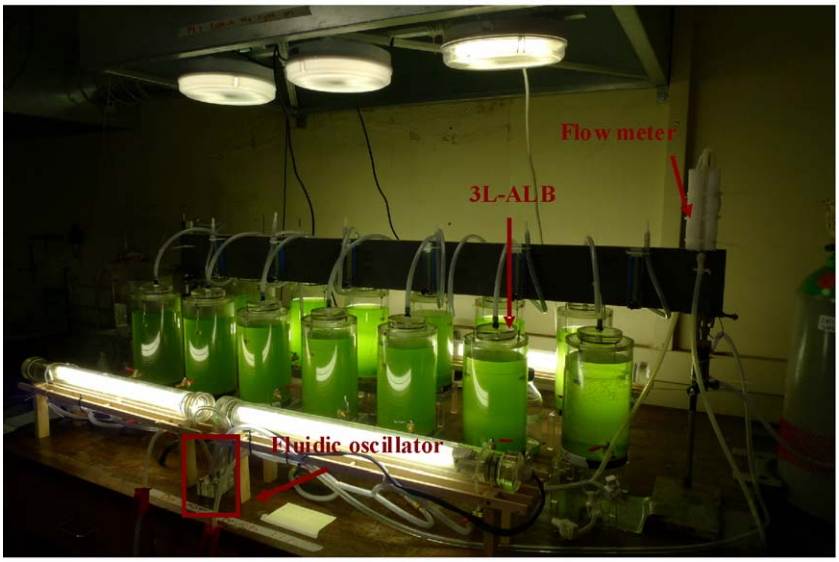

Figure 3. The laboratory setup of 13 ALB bench cultures of *Dunaliella salina*.

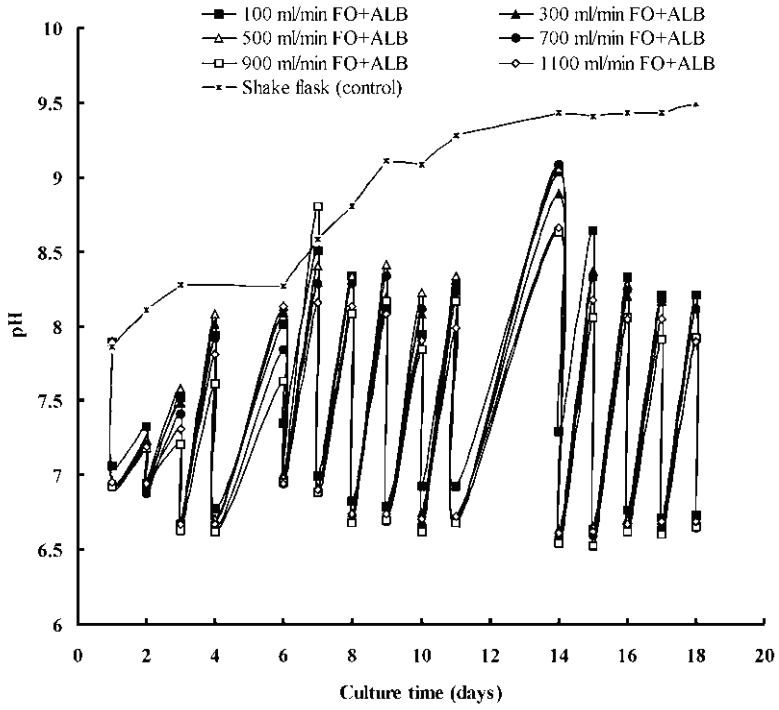

Figure 4. Daily pH changes for ALB and shake flask cultures. For ALB cultures, the higher point and the lower each day represent the pH value before dosing and after dosing, respectively.

decreasing the screening time while using environmental conditions appropriate for full scale production, including stack gas as part of the medium, is more informative and cost effective. Third, about 20-40% improvement of algal overall specific growth rate was found over a wide range of dosing flow rate, comparing FO driven ALB with basic ALB (Figure 6). Impressively, the

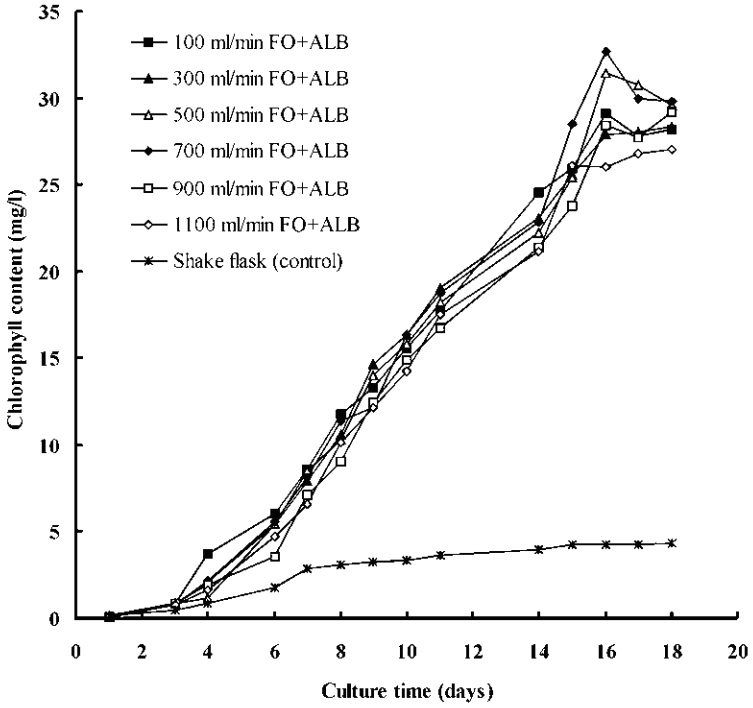

Figure 5. Growth of *Dunaliella salina* in ALBs and shake flask. Chlorophyll content was measured after extraction with 80% acetone.

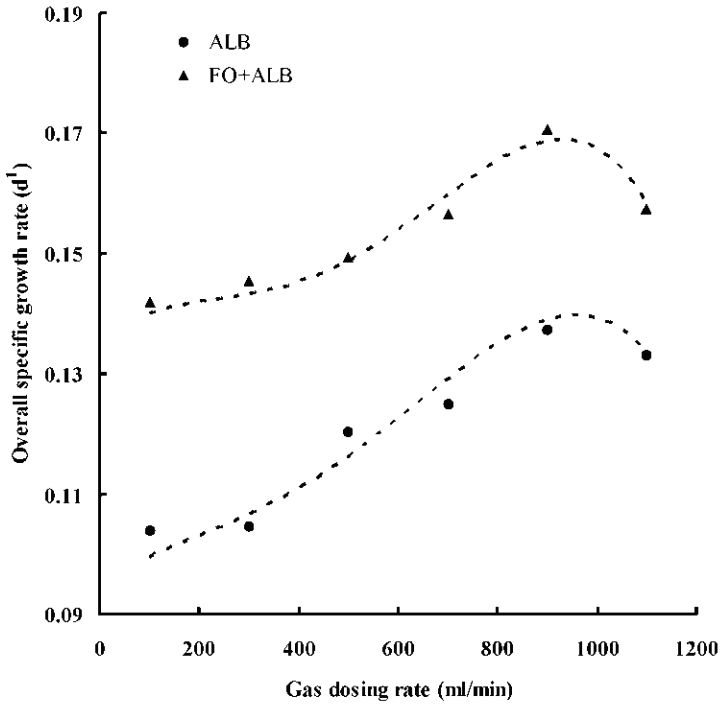

Figure 6. The correlation of overall specific growth rate and dosing flow rate for fluidic oscillator (FO) driven ALBs and basic ALBs.

highest specific growth rate for basic ALB culture was achieved at 0.9 L min^{-1}, whereas the same level was achieved by FO driven ALB at only 0.1 L min^{-1}, which showed a potential large energy saving.

Similarly, Hanotu [15] grew *D. salina* cultures in a 250 litre ALB (Figure 7) and demonstrated about 30% enhancement in yield by induced microbubble dosing, with an overall specific growth rate of 0.13 d^{-1}. In another 250 litre ALB culture with different CO_2 dosing rate, an apparent stripping of oxygen (Kla of 0.13 min^{-1}) was found during microbubble dosing with a similar specific growth rate of 0.11 d^{-1}.

Comparison of FO driven ALB cultures at different CO_2 dosing rates

To compare the performance of FO driven ALB cultures, CO_2 dosing rate was normalized by the equation

$$V_{CO_2} = \frac{U \times CO_2\% \times t_{dosing}}{V_{culture} \times t_{culture}}$$

Where V_{CO_2} represents the CO_2 dosing rate (L L^{-1} d^{-1}), U is the dosing flow rate (L min^{-1}), CO_2% is the carbon dioxide content in the flue gas/mixture gas (%), t_{dosing} and $t_{culture}$ represent total gas dosing time (min) and culture time (d) respectively, $V_{culture}$ shows the culture volume (L).

The correlation between CO_2 dosing rate and algal overall specific growth rate is shown in Table 1. Generally, the overall specific growth rate increases with CO_2 dosing rate in the range of 0.031 - 0.375 L L^{-1} d^{-1}, however further increase in CO_2 dosing rate resulted in a negative effect on algal growth. Specific growth rate (μ) decreases from 0.17 d^{-1} at CO_2 dosing of 0.375 L L^{-1} d^{-1}) to 0.15 d^{-1} at 0.458 L L^{-1} d^{-1}. A similar trend has also been found in published work on PBR cultures of the red alga *Porphyridium* [16] and the diatom *Phaeodactylum tricornutum* [17]. One thing worth noting in Table 1 is that trial 3 and trial 8 are actually out of the trend. For trial 3, due to bigger bubble size, the mass transfer for CO_2 dissolution and O_2 inhibition were most likely limited, therefore, even increasing the dosing rate did not lead to an increase in growth. For trial 8, a higher specific growth rate was achieved than expected, at 2.3 L L^{-1} d^{-1} of CO_2 dosing rate which had exceeded the optimal range. The difference between trial 8 and the others, in terms of operating conditions, is illumination – periodic solar radiation rather than constant artificial lighting. Therefore, it is likely that by introducing constant lighting for laboratory algal cultures photo-inhibition might also be induced, which could lead to a negative effect on growth, while with a high mass transfer dosing (microbubble dosing) and natural illumination, a better growth could be achieved along with energy savings.

Comparison of different types of airlift bioreactors for microalgal culture

There have been a number of other airlift type bioreactors developed for algal biomass production. Table 2 and Table 3 show respectively the operating conditions and outputs reported with different types and scales of airlift reactor cultures. In both a 60 litre split-cylinder airlift reactor and a concentric draft-tube airlift vessel, *P. tricornutum* cultures attained a biomass concentration of about 4 g L^{-1} with maximum specific growth rate of 0.53 d^{-1} after 260 h [18]. In a 200 litre airlift-driven external loop tubular bioreactor, biomass productivity of 1.2 g $L^{-1}d^{-1}$ was achieved at a dilution rate of 0.05 h^{-1} for the same microalga, however, a negative effect of high levels of dissolved oxygen on productivity was detected [19]. In a 13 litre modified airlift bioreactor with helical flow (for *Porphyridium* culture), a lower gas requirement was found than in the other parallel cultures carried out in bubble column and airlift reactors, however, an adverse effect of excessive gas flow rate on growth was also reported [16]. In another study, a bubble column reactor was reported to be preferable for *Dunaliella tertiolecta* culture, rather than an airlift bioreactor - very little algal growth and a significant disruption of the wall-less cells was observed in the airlift reactor [20]. Furthermore, growing *Chlorella vulgaris* in a 3 litre flat panel airlift photobioreactor with intermittent light, led to a maximum biomass productivity of 0.11 g $L^{-1}d^{-1}$ being achieved [21].

It should be noted that in order to compensate for lower mass transfer by conventional bubble dosing, increasing gas flow rate or gas dosing time is commonly considered. However, the negative effect of excessive gas flow rate on algal growth

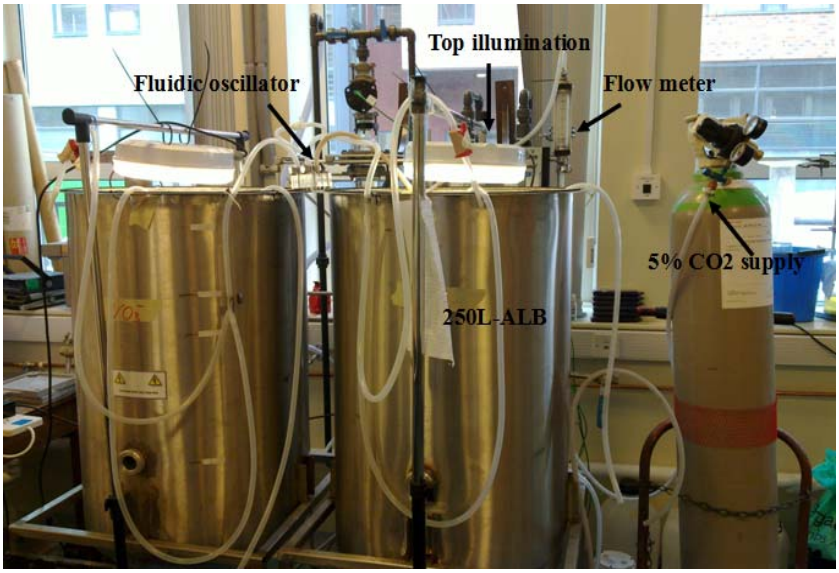

Figure 7. The laboratory setup of the 250 litre ALB culture.

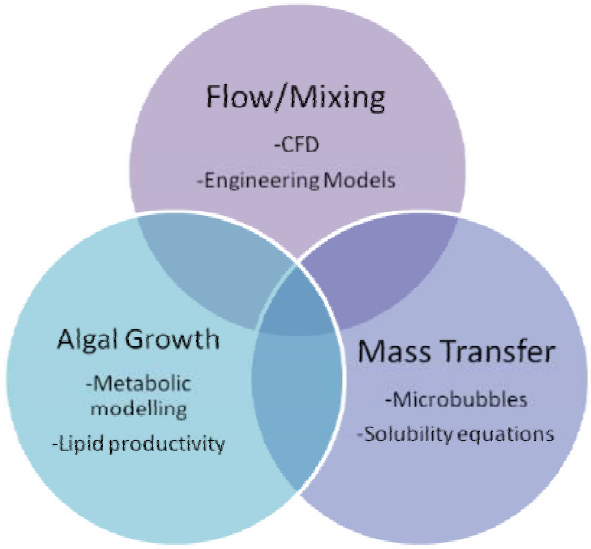

Figure 8. Key modelling components for successful design of algal growth systems.

and the energy cost of constantly supplying gas become obstacles in industrial applications. Besides, for culture systems that achieve good initial biomass productivity, high O_2 level is the crucial limiting factor for further growth. Microbubble-driven airlift loop bioreactor delivers a desirable mass transfer, its relatively low gas requirement with high CO_2 dissolution and O_2 stripping efficiency promises energy saving and CO_2 capture for industrial algal biomass production using flue gas.

Model-based design of algal ponds for optimal productivity

Open and raceway pond systems have been proven as commercially viable systems for the growth of microalgae for many years now [22, 23]. The growth of species such as *D. salina* has made a huge impact on the pharmaceutical and health food industries, due to the cheap mass production of beta carotene and other pigments. This economy of scale has only been possible due to the exploitation of pond designs in conjunction with PBR technology. Recently there has been a reawakening of interest in new designs for open ponds and their aeration systems using models of varying complexity (Figure 8). In this section, we discuss challenges and recent progress towards optimising the cultivation of microalgae in open ponds using novel designs for aeration and mixing.

Features of open pond systems

There are three main types of open pond; circular, raceway and sloping, each with differing benefits and constraints. Their designs typically consist of a series of closed loop flow channels (or a single ‘pond’) that are open to the air. The algal culture

Table 1. Comparisons of FO driven ALB cultures at different CO_2 dosing rates.

Trial	Illumination	Diffuser and bubble size	CO_2 dosing $L_{CO_2}/L_{Culture}/d$	μ d^{-1}	Ref.
1	Constant artificial illumination	Ceramic diffuser 300µm	0.031	0.11	[14]
2			0.125	0.14	[14]
3		Membrane diffuser 500µm	0.18	0.13	[15]
4		Ceramic diffuser 300µm	0.208	0.15	[14]
5			0.292	0.16	[14]
6			0.375	0.17	[14]
7			0.458	0.15	[14]
8	Natural light		2.3	0.22	[10]

is circulated around the pond or raceway circuit by a pump or paddle wheel, to avoid algal flocs forming on the liquid surface and to also provide enhanced aeration of the culture. The depth of the designs is normally around 0.2 - 0.3 meters, to enable greater light penetration into the culture and facilitate mixing. The areas covered can range from 0.5 to 200 hectares, depending on the species selected and the scale required [24].

Although PBRs can provide a greater culture density per unit volume, due to the larger production capabilities and established technology, open pond systems are currently favoured for the potential production of biofuels and other commodity crops. It has been suggested that, in the future a hybrid design, incorporating the best elements from both the pond and reactor systems, may be the most productive. A PBR could produce a large scale, contaminant free inoculum, which could be quickly produced to scale in an open raceway pond before harvesting [24]. Although, algal pond designs have been around for many years and are to some degree optimised, the designs still have many aspects that can cause issues. Contamination is a common problem, due to the open nature of the system, population crashes due to invasion of foreign pathogens and competitors is common, which can affect productivity. Water supply to compensate for evaporative losses, temperature control and levels of insolation during seasonal variations also can have a large impact. However, one of the most overlooked aspects in terms of design optimisation is the mixing and aeration of the cultures.

Mixing and mass transfer

The design of an ideal algal pond is a trade off between optimal hydrodynamic properties and low construction costs (carbon and financial) [25]. Flow resistance due to friction as the media flows over the pond surface is a crucial factor along with the method used for mixing. The higher the frictional loss in the system, the more energy is needed for mixing. Typical pond designs are mixed by paddle wheels, although they are relatively efficient and cheap, they do require consistent maintenance and do not provide very good aeration. To compensate for this, this type of pond often requires additional air sparging to provide the cultures with enough CO_2 to respire effectively. Aeration of the culture, due to the open nature of the pond/raceway also creates other efficiency issues. The CO_2 provided to the culture is expensive unless it is provided on site i.e. flue gas, so the gas must be used efficiently to keep operating costs down and also to allow enough time for mass transfer to take place in the medium. Due to the shallow depth of the ponds, a lot of the gas escapes without been absorbed by the cultures, reducing the absorption efficiency tremendously. Designs with partial covers over the sparging zone have been tried in the past, which did increase the efficiency, but this was at the expense of increased frictional losses.

Design optimisation

Recently there had been a resurgence of interest in pond design spurred on by increased funding from

Table 2. The culture conditions for various airlift bioreactor cultures.

Bioreactor	**Vol.** m^3	**Gas velocity (flow rate)**	**Gas compo-sition**	t_{dosing} h/d	$t_{culture}$ h	**Environmental conditions**	**Nutrients supply**	**Strain**	**Ref.**
Split-cylinder airlift device OR Concentric draft-tube airlift vessel	0.06	0.011 ms^{-1}	N/A	24	260	Light: natural light Temp: 20°C (controlled)	N/A	*Phaeodactylum tricornutum*	[18]
Airlift-driven external-loop tubular photobioreactor	0.2	0.3 ms^{-1}	N/A	N/A	N/A	Light: natural light Temp: 20±2°C (controlled) pH: maintained at 7.7 by auto-injecting CO_2	3 times higher concentration Supplied at dilution rate of 0.05 h^{-1} for 10 h	*Phaeodactylum tricornutum*	[19]
Airlift reactor with helical flow promoters (HFP)	0.013	0.0016 ms^{-1}	3% CO_2	24	216	Light: Luminescent lamps Temperature: 23-25°C	N/A	*Porphyridium* sp.	[16]
Bubble column system	N/A	0.6 ms^{-1}	6% CO_2	24	144	Light: fluorescent lamps Temp: 30°C (controlled)	Optimal medium for *D. tertiolecta*	*Dunaliella tertiolecta*	[20]
Airlift bioreactor	N/A	0.4 ms^{-1}	6% CO_2	24	72	Light: fluorescent lamps Temp: 30°C (controlled)	Optimal medium for *D. tertiolecta*	*Dunaliella tertiolecta*	[20]
Flat panel airlift (FPA) photobioreactor	0.003	N/A	0.45 vvm air	24	120	Light: light intensity gradual adjusted to match the biomass concentration Temp: 29°C (controlled) pH: 6.8±1 (controlled)	Excessive phosphate/nitrate	*Chlorella vulgaris*	[21]
Airlift loop bioreactor (ALB) with fluidic oscillator (FO) – pilot scale	2.2	40 L/min	Flue gas (23% CO_2)	10-12	408	Light: natural light Temp: 23°C (controlled)	*D. salina* medium Without top up	*Dunaliella salina*	[10]
Airlift loop bioreactor (ALB) with fluidic oscillator (FO) – lab scale	0.003	0.1-1.1 L/min	5% CO_2	0.5	432	Light: fluorescent lamps	*D. salina* medium Without top up	*Dunaliella salina*	[14]

Table 3. The output reported in various airlift bioreactors.

Bioreactor	**$C_{biomass}$**	**μ**	**Productivity**	**Estimated % increase in biomass (final conc. – initial conc.)/ initial conc.**	**Ref.**
Split-cylinder airlift device OR Concentric draft-tube airlift vessel	4 g/L	0.53 d^{-1} (Maximal)	N/A	3900%	[18]
Airlift-driven external-loop tubular photobioreactor	2.38 g/L	N/A	1.2 g $L^{-1}d^{-1}$	N/A	[19]
Airlift reactor with helical flow promoters (HFP)	N/A	0.48 d^{-1} (at first day)	N/A	789%	[16]
Bubble column system	N/A	N/A	N/A	1400%	[20]
Airlift bioreactor	Very little algal growth Microscopic examination showed significant disruption of cells				[20]
Flat panel airlift (FPA) photobioreactor	1.95 g/L (dry wt)	N/A	Max: 0.11 g $L^{-1}d^{-1}$	720%	[21]
Airlift loop bioreactor (ALB) with fluidic oscillator (FO) – pilot scale	0.24 g/L (dry wt)	0.22 d^{-1} (overall)	N/A	3500%	[10]
Airlift loop bioreactor (ALB) with fluidic oscillator (FO) – lab scale	N/A	0.14 - 0.17 d^{-1} (at steady growth phase)	N/A	3100% - 3900% (based on Chl)	[14]

various bodies related to sustainable energy alternatives. One system looked at removing the aeration issue from the ponds design by having a separate aeration column that would feed into the pond, based on a similar principle to the degassing columns typically associated with closed PBR systems [26]. Another approach was to increase the aeration sump depth in order to provide mixing and aeration at the same time, thus avoiding the need for a paddle wheel system completely [27]. One potential avenue of improvement in both designs would be the incorporation of microbubbles (as described in earlier sections of this review) to aid in the gas hold-up and mass transfer of respiration gases [28].

Computational modelling

Computer simulations offer an increasingly effective and cheap way to test new design concepts and find optimal design parameters. Designs can be put through numerous development iterations before the need to actually test a physical model, saving time and resources in the process. The relentless increase in hardware performance means that computationally intensive algorithms such as Computational Fluid Dynamics (CFD) can now be run on a laptop. Detailed models incorporating mass transfer, mixing and cell metabolism are now tractable. Attempts to integrate CFD in the algal pond design process have been underway for several years now. Some of the best models have been developed using the US Army Corps of Engineers and EPA codes. The Environmental Fluid Dynamics Code (EFDC) solves 3D Navier-Stokes equations of open channel flow to model speed, temperature, and nutrient gradients. The system also includes solar insolation, climate, meteorological functions and integrates algal biomass growth rates using the CE-QUAL model that couples nutrient kinetics and 22 independent variables (N, P, Si, O_2 etc.) [29]. Simpler models based on preparatory software such as Ansys Fluent and Comsol Multiphysics have also been shown to represent design parameters effectively in conjunction with mass transfer simulation [30]. However, work relating to this has typically been applied to just PBRs so far.

The challenge for the future is to integrate fluid dynamic/mass transfer models with models of algal metabolism and lipid production. This will enable the effects of engineering decisions (aeration rates, pond geometry etc.) to be directly connected to lipid synthesis. Standard methods of mathematical optimisation can then be applied to yield higher algal productivity.

CONCLUSIONS

It is clear that microbubble dosing of ALBs has greatly improved the productivity of microalgae in small scale laboratory experiments (3 litre volume), in larger scale laboratory bioreactors (250 litre volume) and in a 2200 litre bioreactor situated outdoors utilizing flue gas from a steelworks power plant. The results to date suggest that the improved growth of microalgae is due to the avoidance of both CO_2 limitation and O_2 inhibition, which can severely depress the growth rate and productivity of algal cultures. There are applications for this technology in the screening of algal strains for biofuel production and for developing a new generation of photobioreactors based on the ALB/microbubble concept.

In addition, microbubble technology can also be applied to open pond growth systems, such as raceway ponds, to address the relatively neglected area of pond mixing and aeration. It is envisaged that computational modelling will be required to fully understand the mixing parameters of open ponds and allow the microbubble dosing to be tailored to provide gas exchange and mixing in a highly efficient manner.

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