# Chapter 3 Growth Characteristics of Different Algal Species

Sanjeev Mishra and Kaustubha Mohanty

## 3.1 Introduction

Algae are large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multi-cellular forms (Singh and Gu, 2010). Productivity of these photosynthetic microorganisms which converts  $CO_2$  into carbon-rich lipids is only a step or two away from biofuel which in turn is produced by several chemical, biochemical and thermochemical processes (Wijffels and Barbosa, 2010; Kirrolia et al., 2013; Beneroso et al., 2013). Globally algal biofuel has been considered as 3rd and 4th generation biofuel based on its potential over 1st and 2nd generation crop based biofuels. Numerous scientists have discovered various applications of algal biomass apart from biofuel applications for the production of value added products to reduce its production cost towards bio-refinery approach (Rawat et al., 2013). Wijffels and Barbosa (2010) reported in *Science* about the broad prospect of microalgae over terrestrial crop based biofuel. In their report they mentioned how a 50-year-old concept came into focus during the oil crisis of 1970s. Since then over millions of algal species have been isolated, identified and studied towards its potential for biofuel and value added products. Table 3.1 represents microscopic view of some potential algal strains which has been studied as model organism at lab-scale and pilot-scale. Recent studies suggest that green algae are promising species bearing a substantial potential to obtain various products in a biorefinery concept (Suali and Sarbatly, 2012). The algal oil can be transesterified to fatty acid methyl ester (FAME) and non-lipid components of algal biomass such as carbohydrates and proteins can be used for the production of bioethanol, biobutanol, neutraceuticals and

S. Mishra

Centre for Energy, Indian Institute of Technology Guwahati, Guwahati 781039, India

K. Mohanty (🖂)

Department of Chemical Engineering, Indian Institute of Technology Guwahati, Guwahati 781039, India e-mail: kmohanty@iitg.ernet.in

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Microorganisms	Microscopic picture	Microorganisms	Microscopic picture
Scenedesmous sp.		<i>Nitzschia</i> sp.	
<i>Chlorella</i> sp.		Chloromonas sp.	
Nannochloropsis sp.		Botryococcus sp.	
Dunaliella sp.		Chlamydomonas sp.	0
Micractinium sp.	-	<i>Spirulina</i> sp.	A
Ourococcus sp.	00	Anabaena sp.	S.C.
Pavlova sp.		Tetraselmis sp.	

 Table 3.1
 Microscopic view of potential algae

animal feed (Kirrolia et al., 2013). Moreover, the residue biomass cake can be used further to produce liquid fuel using the process of pyrolysis (Beneroso et al., 2013).

Bearing substantial feature towards biorefinery approach, triumphing significant biomass through cost effective process has always been a pronounced challenge (Acién et al., 2012). Amongst the several challenges, isolating and identifying potential algal strains having fast growth rate, high biomass yield, and significant lipid productivity are vital. In identifying potential algal strains, growth characteristics play significant role which further depends on growth medium, growth parameters, cultivation system,  $CO_2$  concentration, algal growth rate, biomass productivity, and many more (Roleda et al., 2013). The current chapter elaborates the growth characteristics of different algal species studied in recent time and their feasibility towards algal biorefinery approach.

# 3.2 Potential Algal Strains

Photosynthetic algae represent an exceptionally diverse but highly specialized group of organisms of which microalgae exists with 1 to 10 million (upper limit) species in the nature (Norton et al., 1996). However, only few have shown the potential to be grown successfully in lab-scale and pilot-scale using photobioreactors and raceway pondss towards production of biofuel and value added products (Wu et al., 2012). Reports suggest that green algae have high prospective towards mass cultivation due to their high growth rate and lipid content which meets the present requirement of algal biorefinery concept (Singh and Gu, 2010). Potential algal strains can be obtained from the repositories present worldwide, but indigenous isolated algal strains will have an inherent adaptability that may be the competitive edge required for mass cultivation systems. In addition, they are able to adapt quickly to the changes in the local environment and climate (Abou-Shanab et al., 2011; Rawat et al., 2013). Indigenous algal strains can be isolated from different aquatic habitats such as pond, lake, river, etc. as well as salt water or brackish water lakes and oceans. Further different microorganism isolation techniques are used to isolate pure algal strains and identified morphologically and genetically. To screen model algal strain for future experiment and mass cultivation studies, those isolate strains undergo several lab-scale experiments such as growth rate estimation, photosynthetic efficiency, nutrient recovery, biomass productivity, lipid yield, lipid profiling etc. (Singh and Gu, 2010).

In recent time, many reports have been published on identifying potential algal strains and their feasibility towards biofuel production. Abou-Shanab et al. (2011) isolated 33 numbers of microalgae strains, from which screened eight strains and studied their potential towards biodiesel production. In evidence to their characteristics study they concluded that *Scenedesmous obliquus* YSR01 was the most potential strain towards biodiesel production. Chaichalerm et al. (2012) isolated six microalgae strains enriched in four different growth mediums for the study of their biomass yield, lipid content, and lipid productivity in batch mode. Based on their

study, Chlorococcum humicola was identified as a potential strain. In contrast to isolating potential strains which can grow in artificial culture medium, there are several strains isolated having the potential to grow in wastewater. These algal strains were proficient towards removing nitrogen and phosphorus as growth nutrients while accumulating lipids in effluents from different domestic and industrial wastewater. Sydney et al. (2011) conducted a screening experiment on 20 algal strains and found positive growth on 13 strains towards nutrient removal and lipid accumulation in secondary domestic wastewater effluents. They concluded that Botryococcus braunii showed highest nutrient removal of 79.63 % on 14 days of cultivation period. Cai et al. (2013) in their review article on "Nutrient recovery from wastewater streams by microalgae: Status and prospects" stated, Chlorella sp. has been widely studied and shown to be effective in nutrient removal and high value biomass production. It has also been claimed that some species of *Chlorella* are heterotrophic or mixotrophic and can consume organic forms of carbon in addition to inorganic nutrients as part of their metabolic process. This can be an advantage when using wastewater streams containing carbon residues, such as digested dairy manure (Wang et al., 2010).

## 3.3 Algal Growth Characteristics

Algae are photosynthetic organisms whose growth characteristics are subjected to light intensity, growth medium (nitrogen, phosphorous, potassium and micronutrients) and carbon dioxide supply (Wang et al., 2010). Since long time many scientists have developed different growth conditions and optimized several growth medium to enhance algae growth rate towards high value biomass yield. Bold's basal medium (BBM), BG-11, TAP and artificial sea water (ASW) are the most common nutrient mediums which have been widely used as the algal (green algae and cyanobacteria) growth medium (He et al., 2013; Basu et al., 2014; Kumar et al., 2014; Bellou and Aggelis, 2012). In recent times, wastewater containing nitrogen and phosphorous as major nutrients has also been widely studied replacing labgrade nutrient medium as low cost cultivation process coupled with wastewater treatment (Cai et al., 2013). Moreover, recent studies suggest that there are several stress effects of nutrients, light, temperature, CO<sub>2</sub> and pH which affect the growth rate and biochemical properties in algal biomass (Suali and Sarbatly, 2012). These stress conditions can be applied during batch mode (single/two-step) or continuous mode (Roleda et al., 2013). As algal cultivation needs to be studied towards biorefinery approach, achieving feasible biomass productivity during mass cultivation has always been a challenge. Acién et al. (2012) in their report suggested using low cost cultivation system in addition to flue gases as carbon source and wastewater as growth medium can help achieving substantial biomass yield up to 4.86 g  $L^{-1}$  (Basu et al., 2014).

## 3.3.1 Methodology to Estimate Algal Growth Rate

Determining algal growth rate is essential in identifying potential algal strains during the screening process and validates its productivity towards mass cultivation prospect. There are several methods through which algal growth rate can be estimated of which specific growth rate and biomass productivity is essential.

#### 3.3.1.1 Spectroscopic Growth Rate Observation

Algae are photosynthetic organisms which contain different pigments. Chlorophyll 'a' and 'b' are among such pigments which is present in green algae and cyanobacterias. Spectrophotometer is used to estimate these pigments and cell concentration in the culture medium. A wide range of wavelength (550–750 nm) is considered to identify algal cell concentration which is estimated at a specific time interval and algal growth curve formed (wave length vs time).

#### 3.3.1.2 Biomass Yield

Besides algal cell concentration, growth rate can also be determined from its biomass growth which is estimated from the total biomass achieved from a specific culture time period. To obtain algal biomass, algal culture is washed repeatedly to remove the nutrients present in it and then moisture-free biomass is obtained through centrifugation followed by drying at approx. 108 °C. There are several kinetic equations available in literatures which were usually used to determine the biomass productivity and yield.

#### 3.3.1.3 Kinetic Equations for Growth Study

Several kinetic equations are reported for algal growth estimation and among them specific growth rate in terms of algal density is quite popular. Besides, dry algal biomass has also been considered to estimate specific growth rate of algae (Xu and Boeing, 2014). ' $\mu$ ' was determined from a linear fit in a semi-logarithmic plot of algal density against time.

$$\dots = \frac{\ln\left(\frac{W_t}{W_0}\right)}{t - t_0} \tag{3.1}$$

In equation (3.1), ' $W_t$ ' is algal biomass concentration (g L<sup>-1</sup>) at the time 't',  $W_0$  is initial biomass concentration (g L<sup>-1</sup>) and 't' is the experimental time (d). Specific growth rate can also be determined from the slope of a semi-log plot of biomass concentration versus time.

## 3.3.2 Growth Medium and Algal Growth Characteristics

Algae are known to have different nutrient requirements not only by composition but also by concentration of the nutrients supplied which is basically composed of macro- and micro-nutrients, dissolved ions, trace metals and several vitamins (Rawat et al., 2013). Growth media for algae are grouped depending on fresh water or salt water species. There is no universal growth media recipe that works for all taxa, so researchers are forced to give great care on how growth media is composed, stored and used. In general, algal growth medium is composed of macro- and micronutrients. Macro-nutrients required by algae, diatoms and cyanobacteria include carbon, nitrogen, phosphorous, silicon and major ions including Na, K, Mg, Ca, Cl and SO<sub>4</sub> as a base media. Micro-nutrients are trace amounts of essential elements and these include iron, manganese, zinc, cobalt, copper, molybdenum and a small amount of metalloid selenium (He et al., 2013).

In the past few decades, tremendous efforts have been put into research of microalgae cultivation in wastewaters for the removal of nitrogen, phosphorus and other elements (Cai et al., 2013). This process of algal bioremediation route towards biomass production makes algal cultivation process cost effective coupled with simultaneous wastewater treatment and biomass production for biofuel and other applications. To pursue algal bioremediation photoautotrophic/photoheterotrophic/ mixotrophic cultivation system were followed. Recently, untreated and treated domestic secondary effluent, municipal wastewater, brewery wastewater, thin stillage, soy whey, carpet industry wastewater and other industrial wastewater were successfully used for microalgal-based bioremediation process.

#### 3.3.2.1 Algal Growth in Conventional Medium

Conventional lab-grade synthetic mediums are widely studied at lab-scale or small scale experiments for screening and process optimization of various algal strains (Abou-Shanab et al., 2011). During the culture process nitrate and phosphate play a central role in microalgal cell physiology and growth where the optimum concentration enhances the growth rate of microalgal cultures (Bhola et al., 2011). In addition to nutrients, CO<sub>2</sub> enhances algal photosynthetic efficiency leading to increase of algal growth rate (Suali and Sarbatly, 2012). In a recent study, Basu et al. (2014) reported indigenous *Scenedesmus obliquus* SA-1 in BG11 medium and studied its growth charac-teristics. *S. obliquus* showed higher biomass concentration of 4.86 g L<sup>-1</sup> which was achieved at  $13.8 \pm 1.5 \%$  CO<sub>2</sub> supply. To study the nitrogen and phosphorous effect on algal growth they increased their concentration and achieved higher biomass concentration of  $4.975 \pm 0.003$  g L<sup>-1</sup> which justified that nitrogen and phosphorous play important role in uplifting algal growth rate.

Algae are known to have specific nutrient requirement for their growth for which their growth characteristics varies at different growth medium. Chaichalerm et al. (2012) grew six algal strain *Chlorococcum humicola*, *Didymocystis bicellularis*,

*Monoraphidium contortum, Oocystis parva, Sphaerocystis* sp., and *Scenedesmus acutus* in four different growth medium (3NBBM, BG-11, Kuhl and N-8) and the mode of algal growth effect was observed by varying nutrient compositions. Significant difference in their growth characteristic and lipid productivity with respect to its growth medium was observed for all the six strains. Among all, *C. humicola* had the highest biomass yield of 0.113 g L<sup>-1</sup>d<sup>-1</sup> (in Kuhl medium), the highest lipid content of 45.94 % w/w (in BG-11 medium), and the highest lipid yield of 0.033 g L<sup>-1</sup>d<sup>-1</sup> (in 3NBBM medium). The 3NBBM medium, which has the lowest nitrogen concentration among the four culture media, was considered the optimal culture medium for *C. humicola* for lipid production. This is due to the fact that nitrogen limitation does not inhibit algal growth and creates stress environment to cell which enhances synthesis of membrane and storage lipid (Cakmak et al., 2014). Some potential algal strains and their growth characteristics have been presented in Table 3.2.

	Specific	Biomass			
	growth rate	productivity (g	Lipid		
Algal strain	$(d^{-1})$	L <sup>-1</sup> )	(%w/w)	References	
Nitzschia cf. pusilla YSR02	$1.68 \pm 0.28$	$1.37 \pm 0.08$	48±3.1	Abou-Shanab et al. (2011)	
Chlorella ellipsoidea YSR03	$1.42 \pm 0.02$	$1.48 \pm 0.04$	32±5.9		
Micractinium pusillum YSW07	1.19±0.17	2.28±0.16	24±0.5	-	
Ourococcus multisporus YSW08	$0.51 \pm 0.14$	$0.95 \pm 0.11$	52±8.3		
Scenedesmus obliquus YSR04	$1.32 \pm 0.05$	1.98±0.04	21±1.1		
Scenedesmus obliquus YSR01	$2.35 \pm 0.55$	$1.57 \pm 0.67$	58±1.5		
Scenedesmus obliquus YSR05	$1.06 \pm 0.03$	$1.75 \pm 0.34$	27±1.9		
Scenedesmus obliquus YSW06	$0.99 \pm 0.02$	1.80±0.13	27±5.6		
Scenedesmus obliquus SA-1	-	4.86	$33.04 \pm 0.46$	Basu et al. (2014)	
Nannochloropsis oculata	0.194–0.571	$\begin{array}{c} 0.296 - 0.497 \\ (g \ L^{-1} d^{-1}) \end{array}$	22.7–41.2	Xu and Boeing (2014)	
Nannochloropsis sp. F&M-M24	-	0.18 (g L <sup>-1</sup> d <sup>-1</sup> )	30.9	Rodolfi et al. (2009)	
Pavlova salina CS 49	-	0.16 (g L <sup>-1</sup> d <sup>-1</sup> )	30.9		
Chlorococcum humicola	-	0.113 (g L <sup>-1</sup> d <sup>-1</sup> )	45.94	Chaichalerm et al. (2012)	

 Table 3.2
 Growth characteristics of different algal strains grown in synthetic media at lab-scale

#### 3.3.2.2 Algal Growth in Wastewater

Wastewater from fertilizer industry, brewery industry, untreated and treated domestic secondary effluent, municipal wastewater, etc. contain high amount of nitrogen, phosphorous and other elements which are major algal growth nutrients. Achieving high nutrient removal along with feasible biomass yield is rather difficult due to high nutrient load in wastewater which creates stress environment for algal growth. There are few algae which has inherent adaptability to sustain their growth in high nutrient load environment. Among them Chlorella sp. are more commonly studied in various types of wastewaters and achieved high nutrient removal in addition to feasible biomass yield (Cai et al., 2013). In a study by Li et al. (2011), isolated Chlorella sp. was grown in municipal wastewater where a higher growth rate of 0.677 d<sup>-1</sup> was achieved without any lag phase. During the 14 d of cultivation period, Chlorella could achieve more than 80 % of nutrient removal (ammonia, total nitrogen, total phosphate) with 0.92 g  $L^{-1}$  of feasible biomass productivity. There is also report which claimed of achieving 9.8 g L<sup>-1</sup> and 6.3 g L<sup>-1</sup> biomass from thin stillage and soy while growing Chlorella vulgaris at mixotrophic conditions in a bioreactor. Besides high biomass yield Chlorella vulgaris could achieve high lipid vield of 43 % and 11 % (w/w) respectively (Mitra et al., 2012).

As mentioned earlier, nutritional requirement for algal growth varies from strain to strain for which different algal strains need to be screened. Sydney et al. (2011) conducted a screening experiment over 20 algal strains on secondary domestic wastewater treated effluents. Among 20 strains three strains-unknown LEM-IM 11, Botryococcus braunii and Chlorella vulgaris-have shown higher growth rate and high nutrient removal. During 2 L photobioreactor study unknown LEM-IM 11 has shown growth rate of 0.19 d<sup>-1</sup> whereas 0.11 d<sup>-1</sup> was observed in *Botryococcus* braunii and C. vulgaris. But greater nutrient removal was achieved by Botryococcus braunii with 79 % nitrate and 100 % phosphate in addition to higher biomass productivity of 0.68 g L<sup>-1</sup> containing over 36.14 % lipid. There are also reports where two different wastewaters were mixed in different proportion to achieve higher algal growth and nutrient removal. In one such study, Scenedesmus obliquus CCAP 276/3A reported a maximum specific growth rate of 0.074 h<sup>-1</sup>, volumetric biomass productivity of 4 mg L<sup>-1</sup> h<sup>-1</sup>, and net biomass generation 0.28 g L<sup>-1</sup> when grown in a culture blend of 25 % (v/v) of urban wastewater from secondary treatment added to 5 % (v/v) olive-oil mill wastewater (Hodaifa et al., 2013). Table 3.3 represents various algal strains grown in different wastewater and their biomass and lipid productivity. This suggests algal strains represent variable growth characteristics for which screening process is highly essential to select potential algal strain to achieve high biomass yield with simultaneous nutrient removal.

S	Source of	Biomass (g	Lipid	Defense
Species	wastewater	L <sup>-1</sup> )	content (%)	References
<i>Chlorella</i> sp.	Thin stillage	$9.8 \pm 0.3$	43	Mitra et al. (2012)
	Soy whey	$6.3 \pm 0.1$	$11.1 \pm 1.1$	
	Domestic secondary effluent	0.42	43	Yang et al. (2011)
	Brewery wastewater	$2.28 \pm 0.09$	$220 \pm 0.02$	Farooq et al.
			$(mg g^{-1})$	(2013)
	Municipal wastewater	1.75	-	Cho et al. (2013)
	Carpet industry	$0.016 \pm 0.003$	$17.00 \pm 2.89$	Chinnasamy et al.
	wastewater	$(g L^{-1} d^{-1})$		(2010)
Scenedesmus obliquus CCAP 276/3A	Blend of 25 % urban wastewater and 5 % olive-oil mill wastewater	0.28 (g dm <sup>-3</sup> )	33.2	Hodaifa et al. (2013)
Dunaliella tertiolecta	Carpet mill untreated wastewater	28 (mg L <sup>-1</sup> d <sup>-1</sup> )	15.20	Chinnasamy et al. (2010)
Botryococcus braunii	Treated domestic sewage	0.68	36.14	Sydney et al. (2011)
Chlamydomonas reinhardtii	Municipal wastewater	$2 (g L^{-1} d^{-1})$	25.25	Kong et al. (2010)
<i>Chlamydomonas</i> sp. TAI-2	Industrial wastewater	1.8	18	Wu et al. (2012)

Table 3.3 Biomass and lipid productivity of different algal strains studied in different waste water

## 3.3.3 Growth Study at Mass Cultivation Prospect

Algal biofuel are globally considered as 3<sup>rd</sup> and 4<sup>th</sup> generation biofuel due to its significant biomass productivity containing high cellular concentration of lipids, resources and economic sustainability and overall potential advantages over other sources of biofuels (Rawat et al., 2013). Productivity of algae, microalgae in general, can be twenty times than that of oil seed crops on per hectare basis and is thus a more viable alternative towards mass cultivation prospect (Chisti, 2007). Moreover, microalgae have faster growth rates than plants and are capable to grow in highly saline waters and utilize a large fraction of solar energy making them effective solar to chemical energy converters (Huber et al., 2006). Various algal strains were studied towards mass cultivation prospect using raceway pond and outdoor photobioreactors (Singh and Gu, 2010). But feasibility over economic viability has always been a greater challenge of algal cultivation at pilot/commercial scale (Acién et al., 2012). There are many vital steps which need to be critically analyzed at each stage such as, isolation of algal strains for mass cultivation prospect which can be either freshwater or marine algae, cultures selected from various culture repositories or indigenous wild types which might be best suited for large scale production (Suali and Sarbatly, 2012). Furthermore, the screening process should identify potential

strains bearing faster growth rate, high biomass yield, and significant lipid productivity. The synergistic interactions that occur between naturally grown algae and other microorganisms cannot be ignored (Rawat et al., 2013). Besides isolation and identification of potential algal strains, reactor design and biomass harvest from mass cultivation system play vital role to make mass cultivation process cost effective (Acién et al., 2012).

#### 3.3.3.1 Raceway Pond

Low cost, low maintenance, easy setup and large volume cultivation capacity make raceway pond more commercially popular among various researchers and industrialists (Suali and Sarbatly, 2012). This is one of the oldest mass cultivation systems which are widely studied for algal biomass production. Though it is commercially popular it has several drawbacks such as wide exposure to environment always contaminates algal culture and can't be recommended during rainy season and winter season with snowfall. Maintaining growth parameters such as temperature, pH and light intensity is also fairly possible. Besides several drawbacks, there are quite a few algal strains which have been successfully grown with a biomass productivity up to 14–50 g m<sup>-2</sup>d<sup>-1</sup>, which can be further enhanced with supply of external CO<sub>2</sub> (Putt et al., 2011). In a recent study by Ranga Rao et al. (2012), Botryococcus brau*nii* has shown high growth rate leading to biomass concentration of 1.8 g  $L^{-1}$  in 18 days of cultivation in a raceway pond having culture capacity of 80 L. Similar biomass concentration (1.9 g L<sup>-1</sup>) with specific growth rate of 0.419 d<sup>-1</sup> was achieved in 18 days of cultivation in 2000 L raceway pond for Botryococcus braunii (Ashokkumar et al., 2014). This growth rate is comparatively four times higher than that observed by Botryococcus braunii in secondary domestic wastewater treatment effluents (Sydney et al., 2011). In another experiment, fast growing marine algae Chlorella variabilis was grown in 400 L raceway pond with a culture volume of 150 L where significant specific growth rate of 0.36 day<sup>-1</sup> was observed. Cultivating the C. variabilis throughout the year, maximum biomass  $(1.76 \pm 0.04 \text{ g L}^{-1})$  was achieved during summer season when high light intensity used to be observed causing higher photosynthesis and algal growth (De Bhowmick et al., 2014). Nurra et al. (2014) studied algal mass cultivation using Nannochloropsis gaditana in a 53 m<sup>3</sup> raceway pond on a biorefinery concept where significant growth rate with biomass productivity of 19.9 g m<sup>-2</sup>d<sup>-1</sup> was achieved. Some of potential algal strain studied towards mass cultivation prospect using raceway pond and photobioreactor are depicted in Table 3.4.

#### 3.3.3.2 Photobioreactor

Controlled growth environment, minimal contamination and high photosynthesis leading to higher biomass yield are promising features of a photobioreactor as an algal cultivation system. Among several photobioreactor designs, stirred tank and

Cultivation system	Species	Biomass (g L <sup>-1</sup> )	Lipid content (%)	References
Raceway pond	Botryococcus braunii	2.31	-	Ranga Rao et al. (2012)
	Botryococcus braunii	1.9	48.40	Ashokkumar et al. (2014)
	Chlorella variabilis	1.76±0.04	-	De Bhowmick et al. (2014)
	Spirulina platensis	13.5 (g <sup>-2</sup> d <sup>-1</sup> )	-	Jiménez et al. (2003)
	Anabaena sp. ATCC 33047	23.5 (g <sup>-2</sup> d <sup>-1</sup> )	-	Moreno et al. (2003)
	Nannochloropsis gaditana	19.9 (g m <sup>-2</sup> d <sup>-1</sup> )	22	Nurra et al. (2014)
Photobioreactor	Nannochloropsis sp. F&M-M24	$\begin{array}{c} 0.30 \ (g \ L^{-1} \\ d^{-1}) \end{array}$	60	Rodolfi et al. (2009)
	Tetraselmis suecica	$\begin{array}{c} 0.35 \pm 0.03 \text{ g} \\ L^{-1} \text{ d}^{-1} \end{array}$	-	Michels et al. (2014)
	Chlorella zofingiensis	58.4 mg L <sup>-1</sup> d <sup>-1</sup>	54.5	Feng et al. (2011)
	Chlorella sp.	4.3 g L <sup>-1</sup> d <sup>-1</sup>	-	Doucha and Lívanský (2009)
	Botryococcus braunii	2.31	-	Ge et al. (2011)

Table 3.4 Growth characteristics of different algal strains at mass cultivation prospect

vertical column photobioreactors are commonly used during lab-scale experiments, whereas high volume flat panel and tubular reactors are recommended during pilot-scale experiment (Wang et al., 2012). Due to controlled growth parameters most algal strains showed better growth rate and delivers higher biomass yield. *Botryococcus braunii* achieved significant growth rate when grown in photobioreactor and resulted 2.31 g L<sup>-1</sup> of biomass on 25<sup>th</sup> day of cultivation with 20 % v/v CO<sub>2</sub> which was supplied in the form of flue gas (Ge et al., 2011). This is comparatively higher than the biomass obtained from raceway pond (Ranga Rao et al., 2012). Since algal growth rate is specific to strains and its potential towards mass cultivation prospect, research has been focused on cultivating potential high biomass productivity algal strains with a mass cultivation prospect.

Doucha and Lívanský (2009) cultivated fast growing *Chlorella* sp. in outdoor open thin-layer photobioreactor having culture volume of 2000 L where greater biomass productivity was achieved. During this maximum algal concentration of 4.3 g  $L^{-1}d^{-1}$  was achieved whereas less growth rate was observed by *Chlorella zofingiensis* grown in 60 L flat plate photobioreactor. The highest specific growth rate and biomass productivity obtained was 0.994 d<sup>-1</sup> and 58.4 mg  $L^{-1}d^{-1}$  respectively (Feng et al., 2011). Besides fresh water algal strains, sea water and brackish water algal strains have also been studied on scale-up experiments using photobioreactors. High lipid producing *Nannochloropsis* sp. F&M-M24 has shown significant growth

rate and average biomass productivity of 0.30 g L<sup>-1</sup>d<sup>-1</sup> when grown in 110 L Green Wall Panel photobioreactors under nutrient sufficient and deficient conditions containing over 60 % w/w lipid (Rodolfi et al., 2009). Similar growth rate was observed from *Tetraselmis suecica* of  $0.35 \pm 0.03$  g L<sup>-1</sup>d<sup>-1</sup> with optimum biomass concentration of 0.7 g L<sup>-1</sup> when grown in 40 L tubular photobioreactor (Michels et al., 2014). This suggests fresh water green algae have high potential towards mass cultivation prospect using photobioreactor.

## 3.4 Conclusion

Recent reports in the field of algal research suggest different algal species bear different growth characteristic which depends on their mode of cultivation in addition to medium of nutrition and  $CO_2$  supply. Over several algal species, green algae have shown promising growth characteristics and have been widely studied. Potential green algae have shown sustainable growth in different wastewater with respect to high nutrient removal efficiency which makes algal cultivation process a cost effective process when coupled with simultaneous wastewater treatment. Fresh water algal strains have shown significant growth features towards mass cultivation prospect over saline water algal strains. However, nutritional stress can result in high lipid yield from algal biomass during mass cultivation process which has feasibility towards algal biorefinery concept.

## References

- Abou-Shanab, R.A.I., Hwang, J.-H., Cho, Y., Min, B. and Jeon, B.-H. (2011). Characterization of microalgal species isolated from fresh water bodies as a potential source for biodiesel production. *Appl. Energy*, 88, 3300–3306.
- Acién, F.G., Fernández, J.M., Magán, J.J. and Molina, E. (2012). Production cost of a real microalgae production plant and strategies to reduce it. *Biotechnol. Adv.*, 30, 1344–1353.
- Ashokkumar, V., Rengasamy, R., Deepalakshmi, S., Sivalingam, A. and Sivakumar, P. (2014). Mass cultivation of microalgae and extraction of total hydrocarbons: A kinetic and thermodynamic study. *Fuel*, 119, 308–312.
- Basu, S., Roy, A.S., Mohanty, K. and Ghoshal, A.K. (2014). CO<sub>2</sub> biofixation and carbonic anhydrase activity in *Scenedesmus obliquus* SA1 cultivated in large scale open system. *Bioresour*. *Technol.*, 164, 323–330.
- Bellou, S. and Aggelis, G. (2012). Biochemical activities in *Chlorella* sp. and *Nannochloropsis* salina during lipid and sugar synthesis in a lab-scale open pond simulating reactor. J. Biotechnol., 164, 318–329.
- Beneroso, D., Bermúdez, J.M., Arenillas and Menéndez, J. (2013). Microwave pyrolysis of microalgae for high syngas production. *Bioresour. Technol.*, 144, 240–246.
- Bhola, V., Desikan, R., Santosh, S.K., Subburamu, K., Sanniyasi, E. and Bux, F. (2011). Effects of parameters affecting biomass yield and thermal behavior of *Chlorella vulgaris*. J. Biosci. Bioeng., 111, 377–382.
- Cai, T., Park, S.Y. and Li, Y. (2013). Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renew. Sustain. Energy Rev.*, 19, 360–369.

- Cakmak, Z.E., Olmez, T.T., Cakmak, T., Menemen, Y. and Tekinay, T. (2014). Induction of triacylglycerol production in *Chlamydomonas reinhardtii*: Comparative analysis of different element regimes. *Bioresour. Technol.*, 155, 379–387.
- Chaichalerm, S., Pokethitiyook, P., Yuan, W., Meetam, M., Sritong, K., Pugkaew, W., Kungvansaichol, K., Kruatrachue, M. and Damrongphol, P. (2012). Culture of microalgal strains isolated from natural habitats in Thailand in various enriched media. *Appl. Energy*, 89, 296–302.
- Chinnasamy, S., Bhatnagar, A., Hunt, R.W. and Das, K.C. (2010). Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. *Bioresour. Technol.*, 101, 3097–3105.
- Chisti, Y. (2007). Biodiesel from microalgae. Biotechnol. Adv., 25, 294-306.
- Cho, S., Lee, N., Park, S., Yu, J., Luong, T.T., Oh, Y.-K. and Lee, T. (2013). Microalgae cultivation for bioenergy production using wastewaters from a municipal WWTP as nutritional sources. *Bioresour. Technol.*, 131, 515–520.
- Bhowmick, G., Subramanian, G., Mishra, S. and Sen, R. (2014). Raceway pond cultivation of a marine microalga of Indian origin for biomass and lipid production: A case study. *Algal Res.* DOI: 10.1016/j.algal.2014.07.005.
- Doucha, J. and Lívanský, K. (2009). Outdoor open thin-layer microalgal photobioreactor: Potential productivity. J. Appl. Phycol., 21, 111–117.
- Farooq, W., Lee, Y.-C., Ryu, B.-G., Kim, B.-H., Kim, H.-S., Choi, Y.-E. and Yang, J.-W. (2013). Two-stage cultivation of two *Chlorella* sp. strains by simultaneous treatment of brewery wastewater and maximizing lipid productivity. *Bioresour. Technol.*, 132, 230–238.
- Feng, P., Deng, Z., Hu, Z. and Fan, L. (2011). Lipid accumulation and growth of *Chlorella zofingi*ensis in flat plate photobioreactors outdoors. *Bioresour. Technol.*, 102, 10577–10584.
- Ge, Y., Liu, J. and Tian, G. (2011). Growth characteristics of *Botryococcus braunii* 765 under high CO<sub>2</sub> concentration in photobioreactor. *Bioresour. Technol.*, 102, 130–134.
- He, P.J., Mao, B., Lü, F., Shao, L.M., Lee, D.J. and Chang, J.S. (2013). The combined effect of bacteria and *Chlorella vulgaris* on the treatment of municipal wastewaters. *Bioresour. Technol.*, 146, 562–568.
- Hodaifa, G., Sánchez, S., Martínez, M.E. and Órpez, R. (2013). Biomass production of *Scenedesmus obliquus* from mixtures of urban and olive-oil mill wastewaters used as culture medium. *Appl. Energy*, 104, 345–352.
- Huber, G.W., Iborra, S. and Corma, A. (2006). Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. *Chem Rev*, 106, 4044–4098.
- Jiménez, C., Cossío, B.R. and Niell, F.X. (2003). Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: A predictive model of algal yield, *Aquaculture*, 221, 331–345.
- Kirrolia, A., Bishnoi, N.R. and Singh, R. (2013). Microalgae as a boon for sustainable energy production and its future research and development aspects. *Renew. Sustain. Energy Rev.*, 20, 642–656.
- Kong, Q-X., Li, L., Martinez, B., Chen, P. and Ruan, R. (2010). Culture of microalgae *Chlamydomonas reinhardtii* in wastewater for biomass feedstock production. *Appl Biochem Biotechnol.*, 160, 9–18.
- Kumar, K., Dasgupta, C.N. and Das, D. (2014). Cell growth kinetics of *Chlorella sorokiniana* and nutritional values of its biomass. *Bioresour. Technol.*, 167, 358–366.
- Li, Y., Chen, Y.-F., Chen, P., Min, M., Zhou, W., Martinez, B., Zhu, J. and Ruan, R. (2011). Characterization of a microalga *Chlorella* sp. well adapted to highly concentrated municipal wastewater for nutrient removal and biodiesel production. *Bioresour. Technol.*, 102, 5138–5144.
- Michels, M.H.A., Slegers, P.M., Vermuë, M.H. and Wijffels, R.H.(2014). Effect of biomass concentration on the productivity of *Tetraselmis suecica* in a pilot-scale tubular photobioreactor using natural sunlight. *Algal Res.*, 4, 12–18.
- Mitra, D., van Leeuwen, J. (Hans) and Lamsal, B. (2012). Heterotrophic/mixotrophic cultivation of oleaginous *Chlorella vulgaris* on industrial co-products. *Algal Res.*, 1, 40–48.

- Moreno, J., Vargas, M.A., Rodri'guez, H., Rivas, J. and Guerrero, M.G. (2003). Outdoor cultivation of a nitrogen-fixing marine cyanobacterium, *Anabaena* sp. ATCC 33047. *Biomol. Eng.*, 20, 191–198.
- Norton, T.A., Melkonian, M. and Anderson, R.A. (1996). Algal biodiversity. *Phycologia*, 35, 308–326.
- Nurra, C., Torras, C., Clavero, E., Ríos, S., Rey, M., Lorente, E., Farriol, X. and Salvadó, J. (2014). Biorefinery concept in a microalgae pilot plant: Culturing, dynamic filtration and steam explosion fractionation. *Bioresour. Technol.*, 163, 136–142.
- Putt, R., Singh, M., Chinnasamy, S. and Das, K.C. (2011). An efficient system for carbonation of high-rate algae pond water to enhance CO<sub>2</sub> mass transfer. *Bioresour Technol.*, 102(3), 3240–3245.
- Rao, R., Ravishankar, G. and Sarada, R. (2012). Cultivation of green alga *Botryococcus braunii* in raceway, circular ponds under outdoor conditions and its growth, hydrocarbon production. *Bioresour. Technol.*, 123, 528–533.
- Rawat, I., Ranjith Kumar, R., Mutanda, T. and Bux, F. (2013). Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Appl. Energy*, 103, 444–467.
- Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G. and Tredici, M.R. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnol. Bioeng.*, 102, 100–112.
- Roleda, M.Y., Slocombe, S.P., Leakey, R.J.G., Day, J.G., Bell, E.M. and Stanley, M.S. (2013). Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. *Bioresour. Technol.*, 129, 439–449.
- Singh, J. and Gu, S. (2010). Commercialization potential of microalgae for biofuels production. *Renew. Sustain. Energy Rev.*, 14, 2596–2610.
- Suali, E. and Sarbatly, R. (2012). Conversion of microalgae to biofuel. *Renew. Sustain. Energy Rev.*, 16, 4316–4342.
- Sydney, E.B., da Silva, T.E., Tokarski, A., Novak, A.C., de Carvalho, J.C., Woiciecohwski, A.L., Larroche, C. and Soccol, C.R. (2011). Screening of microalgae with potential for biodiesel production and nutrient removal from treated domestic sewage. *Appl. Energy*, 88, 3291–3294.
- Wang, B., Lan, C.Q. and Horsman, M. (2012). Closed photobioreactors for production of microalgal biomasses. *Biotechnol. Adv.*, 30, 904–912.
- Wang, L., Li, Y., Chen, P., Min, M., Chen, Y., Zhu, J. and Ruan, R.R. (2010). Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae *Chlorella* sp. *Bioresour. Technol.*, 101, 2623–2628.
- Wijffels, R.H. and Barbosa, M.J. (2010). An outlook on microalgal biofuels. *Science*, 329, 796–799.
- Wu, L.F., Chen, P.C., Huang, A.P. and Lee, C.M. (2012). The feasibility of biodiesel production by microalgae using industrial wastewater. *Bioresour. Technol.*, 113, 14–18.
- Xu, Y. and Boeing, W.J. (2014). Modeling maximum lipid productivity of microalgae: Review and next step. *Renew. Sustain. Energy Rev.*, 32, 29–39.
- Yang, J., Li, X., Hu, H., Zhang, X., Yu, Y. and Chen, Y. (2011). Growth and lipid accumulation properties of a freshwater microalga, *Chlorella ellipsoidea* YJ1, in domestic secondary effluents. *Appl. Energy*, 88, 3295–3299.