

**Note to Editor:**

**Microalgae cultivation for wastewater treatment and biofuel production: a bibliographic overview of past and current trends**

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Rigorous interest in the quality of surface waters and the related field of treatment of municipal and industrial wastewaters is not novel. Standards to protect environmental quality were developed by the UK Royal Commission on Sewerage Disposal in 1898 [1]. However related research activities become more obvious in the peer-reviewed publication record after the late 1960's [2, 3] reflecting the industrial and urban expansion of the times and the increasing awareness of the impact on surface water. This created the impetus for regulatory authorities to introduce environmental controls on water quality and on urban and industrial emissions. The creation by US EPA of the Clean Water Act of 1972, designed to regulate the restoration and to uphold the quality of the water sources in the United States, is such an example. Related regulations on water discharge stimulated investigations on effective means of nutrient removal, primarily N and P, including the option of microalgae, to mitigate eutrophication of surface waters [2]. Nevertheless, the use of algae to treat wastewaters for reduction of nutrients and biological oxygen demand

(BOD) has long been considered as an effective alternative to conventional biological wastewater treatment processes, to achieve environmental quality standards [2, 4]. Significant peer reviewed literature targeting the use of [micro]algae as an option for wastewater treatment can be traced to about 1977, and, although mentioned before [5], the first clear statement on the value of wastewater for algal production appeared in 1979 [6]. Subsequently, US national programs aimed at developing algal based biofuels also integrated wastewater research elements, a trend especially evident after 1980 [7, 8]. Other bio-products, such as ethanol from residual starches, residual protein for animal feed, nutraceuticals, or even bioplastics may be also be obtained from algal residues left behind after the extraction of lipids for biofuel [8]. The significant nutrient demand of large-scale algae biomass production also provided the opportunity to couple the treatment of high nutrient content wastewaters with algal growth [6, 7]. An additional benefit of wastewater treatment with algae is the capacity to fix CO<sub>2</sub> [9-12]. Biological nutrient removal from wastewater by a range of algal species is effective in a variety of engineered systems including traditional ponds, high rate algal ponds (HRAP [13]). By combining wastewater treatment with algal biofuel production, biological wastewater treatment processes, which are usually a significant energy sink, can be converted into a positive energy source [8, 14].

Therefore, in recent years, research has been devoted to enhancing efficiency of the process of creating biofuels from wastewater derived algal biomass. While other valuable bio-products can and are also obtained from wastewater cultured algae, often from the same harvest [8], the principal driver of our review is the production of biofuels. Literature reviews regularly published on the subject are often written as expert opinions, an approach intrinsically selective. Here we assessed the current state of the science as published, by analysing keyword datasets descriptive of peer-reviewed publications as summarised by a publicly available curated database. By not relying on

an expert opinion approach, we did not select results based on their perceived quality; articles were retained if they met the respective search criteria, and thus their contribution to the keyword dataset was not-biased by a quality judgment. We then examined apparent historical trends in research on the application of algae in wastewater treatment to possibly identify critical research priority areas. Methodological details can be found in the Supplementary data.

*Variation in publications counts:* The annualized rate of increase in publication counts can be used to reveal the maturity of a research field. A mature research area, such as “water” or “algal research”, while producing many publications, has a small proportional rate of increase in publication counts from year to year. Interestingly, the broad topic of using algae for wastewater treatment in general, follows the behaviour of a relatively mature field despite the comparatively smaller publication count (Figure 1a). On the other hand, the large annualized increase rates in manuscript counts for algae for biofuel production, with or without wastewater, suggest a new and expanding field. This is confirmed by the similar trend observed in publication rate for wastewater/algae (wwt/a) and wastewater/algae/biofuels (wwt/a/bf), with the latter a 20% subset of wwt/a (Figure 1b). This trend was consistent irrespective of the type of wastewater type considered (municipal, industrial, and farm wastewater streams) for either treatment or biofuel production.

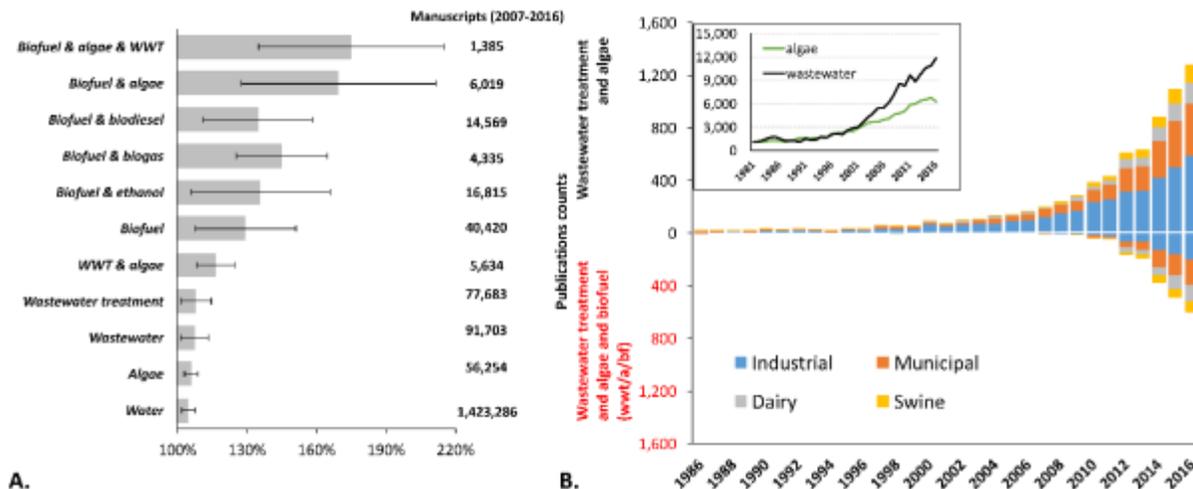


Figure 1. Publications for selected research areas (SCOPUS search results obtained on January 18, 2017); error bars are 95% Confidence Intervals); Manuscript counts sum the period from 2007 through 2016. A. Publications; average annual increase rate and total (2007-2016); B. Publications related to algae and biofuels across wastewater types

*Keyword utilization patterns:* The variation in keyword usage intensity conjectures the rationale and context of the associated research area. The analysis confirms that early interest in wastewater treatment was driven primarily by environmental concerns (Figure 2) with less focus on utilization of wastewaters for resource recovery as substrates in bioreactor or like systems. Thus, environmental impact keywords were identified in about 50% of the 1972-1973 related publications (Figure 2). This was followed by a sustained increase in modelling efforts, likely summarizing the extensive modelling of wastewater treatment carried out by the profession of Civil Engineering [3]. It is interesting to also note the sustained and simultaneous increase of environmental impact and modelling research in the 1990-2000 period (Figure 2).

Wastewater treatment aims to lower BOD and remove nutrients to minimize eutrophication risks [4]. It is noteworthy that the pollution focus of the wwt/a publications is also associated with a significantly stronger focus on metal and toxicity terminology (see Supplementary data, Table 6); average abundance for the keywords subsets including As, Cd, Cr, Cu, Ni, Zn, “metals” and “metal

ions” was  $1.36\% \pm 0.69\%$  for wastewater (wwt) and  $4.39 \pm 1.66\%$  for wwt/a; none were found in the wwt/a/bf publications dataset. This strengthens the notion that addition of algae to the wastewater treatment technologies was initially done with the goal of treatment and not for obtaining algal bio-products.

After 2000 “modelling” dominates the wwt publications (11.5%), “management” and “water pollutants/pollution” are comparably represented in the wwt/a publications (20.1% and 19.0%, respectively), and “biomass”, at 72.8%, clearly dominates the ww/a/bf publications. Nevertheless, research on modelling of wastewater systems, while relatively constant from 1970 through early 2000’s, declined in the last 10 years. This underlying trend, that occurred while publication in the ww/a/bf research area accelerated, is a significant concern. It suggests that much of the recent research is exploratory in scope and likely narrative in nature. Therefore, the development of coherent management tools for algal wastewater treatment processes might be justifiably considered as a priority area for future research investment;

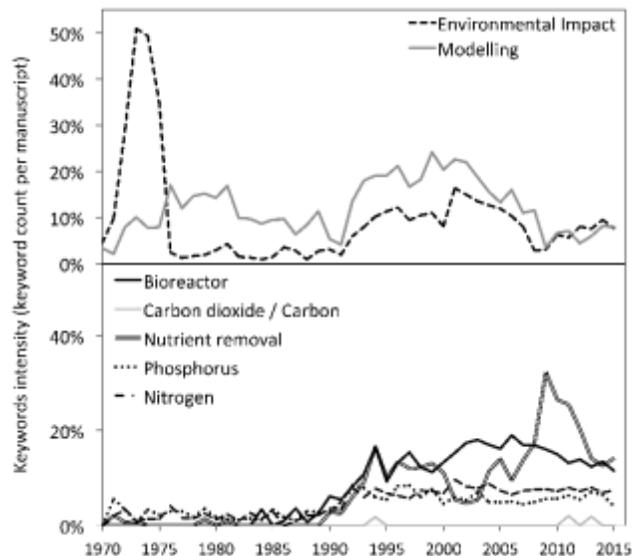


Figure 2. Utilization rates for selected keywords for the “wastewater treatment” query (SCOPUS search results obtained on May 18, 2016)

Where wastewaters are primarily employed for algal growth and biomass production the availability of nutrients becomes a critical aspect of the treatment system. More recently “nutrients” and “nutrient removal”, in the context of algal biofuel, have received greater attention by the international research community, and, concomitantly, bioreactor based research has also expanded; the increased intensity of keywords describing bioreactor type (Figure 2a) towards 1996 coincides with the conclusion of the first concerted effort to evaluate the utility of algae for energy production [7]. The intensity of research on nitrogen and phosphorus, in general, follows a similar trend; research on nutrient removal reached its maximum intensity in 2010, coinciding with a significant output of wwt/a/bf research (Figure 2) in the middle of the current surge in wastewater and algae for biofuel research [8]. A closer look at keyword abundance after 2000 shows that while “nutrient removal” dominates (28.1% for wwt/a, and 17.5% for wwt/a/b), “nutrient availability” or “uptake” received very little attention (0.97% and 0.78%, for wwt/a and respectively wwt/a/bf, and not present in the wwt dataset; Supplementary data, Table 6). This confirms that, whilst nutrient removal, i.e. wastewater treatment, was the key focus of research, the interest in use of wastewater as a nutrient source was only establishing. The increasing use of “nutrient” for the wwt/a/bf literature (18.87%, versus 1.75% for wwt, and 9.38% for wwt/a) also indicated a shift in the approach, but the direction was more difficult to gauge without a qualifier term. These trends suggest that despite the increase in research on wastewater usage for algal production the dominant paradigm surrounding wastewater nutrients is still treatment, i.e. the capacity of algae to remove nutrients from wastewaters, and only secondarily the capacity of wastewater to support algal growth, yet not necessarily optimal growth.

The total number of distinct keywords, or keyword richness, increases as the scope of a given research field expands. All three areas of research, wwt, wwt/a and wwt/a/bf, show an increased

in keyword richness to reach relatively similar level in 2015 (Figure 3a). For the more established wwt and wwt/a research an average annual increase of approximately  $0.5 \text{ keywords y}^{-1}$  is recorded. On the other hand, wwt/a/bf showed a rapid increase in distinct keywords at a rate of  $2.3 \text{ y}^{-1}$ , consistent with a rapidly expanding research field. This pattern is consistent with the early stages of a newly establishing field as shown by the similar rapid increase in keywords of  $3.95 \text{ y}^{-1}$  in the early period of wwt research (1970-1978). Patterns in keyword richness may also reveal when research areas diversify into new directions; this was evident by the patterns observed for wwt and wwt/a around year 2000. Consequently, the dataset was divided to take this behaviour into account to allow a more critical analysis of the patterns and trends in research to be scrutinized after 2000.

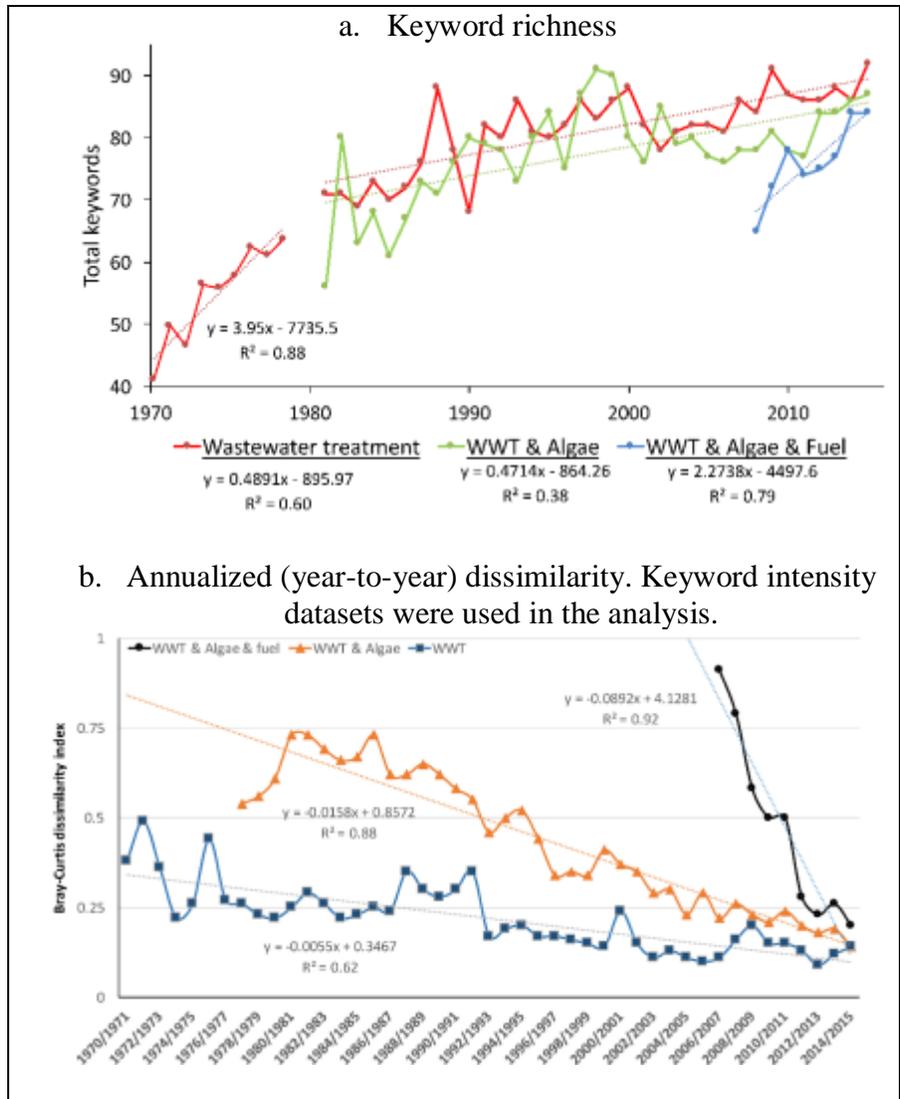


Figure 3 Temporal shifts in keyword utilization (SCOPUS search results obtained on May 18, 2016)

Another indicator of a maturing research field is the stabilization of the range of inquiry. As a field of research shifts from discovery to application the diversity of keywords, and thus the range of inquiry, tends to stabilize. A Bray-Curtis dissimilarity index analysis was therefore applied to assess the year-over-year changes in similarity between the annual keyword datasets to assess the running dissimilarity (Figure 3b). The Bray-Curtis index considers both presence and abundance

for computation of similarity distances. The index declines with time for all three research areas. The rate of decrease is expectedly greater for faster maturing fields.

Values of dissimilarity above the long-term average trend (i.e. the linear fit line) indicated either that: (1) there was a slower decrease in dissimilarity or, (2) there was an increase in dissimilarity for the pair of years in comparison to the previous period. The second condition applies, for instance, when a set of newly added keywords is significantly different from the keywords found in the previous year. Such patterns in the use of different keywords are indicative of an increase in the scope of research in that area, possibly reflecting a period of innovative development. On the other hand, dissimilarities lower than the multiannual trend indicate a relative stagnation in the scope of research, or more stable, less innovative, research activity. For all three areas of research evaluated here, wwt, wwt/a, and wwt/a/bf, there was a consistent decrease in the year-over-year dissimilarity values, which may reflect the relative decrease in innovation, an indicator of the research scope in these areas reaching a certain steady state. A principal component analysis confirmed that research on biomass production parameters increased since 2000. *Chlorella* spp. employed for algal research, including cultivation, for over a century [15, 16] still dominate as the preferred test organisms (see Supplementary data).

A survey of published review articles shows that interest in large scale cultivation of algae can be traced to the 1940s; a monograph published in 1953 summarised much of the state of the art research on algal cultivation from laboratory to pilot scale, with a focus on *Chlorella* spp. [15]; the justification of this work was the potential for algae as food source. Research on sewage for cultivation of microalgae followed soon after [17]. In 1978 the Aquatic Species Program [7], an US national funded activity, identified the potential of producing biofuels through algae and

microalgae cultivation. In 1979 Beneman et al. [6] also published a conceptual map for the use of wastewater to culture algae on wastewaters for fuel production. Much of the initial focus was on hydrogen production and, subsequently, biodiesel production became more important after 1980. The program was terminated in 1996, but in 2010, a new algae for biofuel program was established [8] that also included integration with water treatment facilities. A query for reviews with the keywords “algae” (including “microalgae” and different spellings) and “wastewater” produced a dataset of 230 reviews. These reviews were examined and only those focusing on growth of algae in wastewater were retained. Reviews dealing with the impacts of wastewaters on the environment and on algal blooms in water bodies, and general wastewater treatment or biosorption reviews were excluded. Just under 80 reviews were identified as relevant to biofuels from algal biomass cultivated in wastewater (as listed by SCOPUS on March 16, 2016). The bulk of the reviews, many with a (bio)fuel perspective, were published after 2010 and generally focus on the parameters affecting algae production in wastewater from an engineering perspective.

The first review identified considering the growth of algae in wastewater, from a biotechnology perspective, was published in 1997 [18]. This examined the use of microalgae for bio-treatment and by-products with an emphasis on *Chlamydomonas reinhardtii*. The majority of reviews focused on both production and harvesting of algae [9, 11, 12, 19-44] whereas other focus specifically on harvesting issues [45, 46]. Several articles after 2010 review biodiesel production [20, 25, 37] and the effect of light source in bioreactor cultures, although not necessarily for wastewater based systems [47, 48].

Many reviews [9, 11, 18, 20-24, 26, 27, 31-35, 41-43, 46, 49-58] consider algae in wastewater treatment systems as a biorefinery strategy considering a range of organic compounds, not only lipids. For example, Makrou et al. highlighted the potential production of carbohydrate by algae

as an approach to biosynthesising biofuels [59]. Several reviews, after 2014, cover related areas of research on algal biofilms for wastewater systems and biofuel production [60-62], indicating the rapid development of the field and that it is an area attracting interest from different research groups internationally. The application and development of synthetic biology technologies in algal-based bioconversion systems has also received attention [20, 23, 25-28, 32, 33, 51, 56, 63, 64]. Impacts of large-scale cultures on environmental governance [65], environment [66, 67], resource management (specific to China [68]) and financial viability [67, 69] have also been examined.

By contrast, relatively little attention has focussed on the role of algal biodiversity [63] with *Chlorella* spp. confirmed as still the most dominant test species for algal growth as indicated by two reviews from 2013 and 2015 [53, 58]. Many studies on algal growth have been performed with artificial media, however a range of wastewaters have also been investigated to reflect conditions more realistic of operational circumstances [68].

More dynamic understanding of algal growth processes and behaviour has been gained through the investigation of biological mechanisms and management systems and their combined impact on process performance, as illustrated by reviews summarizing the interactions among algae [70, 71], with microbial consortia [19, 38, 50, 53], and particularly with wastewater associated microbes [72, 73]. Consortia of microalgae, compared to single species cultures, are also shown to be advantageous for productivity and biomass stability [71, 74]. Several recent reviews advocated mixotrophic cultivation to enhance biomass productivity [56, 57, 74, 75], and two-stage cultivation, with a luxury consumption stage followed by nitrogen limitation, is recommended for lipid production [62, 74, 75]. Both nutrient removal [76-79] and nutrient uptake [36, 80, 81] are discussed in the context of lipid production.

Our exploratory analysis, shows that growing algae for biofuel on wastewater substrates is a rapidly expanding area of research, with a comprehensive approach extending beyond the conventional scientific disciplines commonly associated with wastewater treatment. However, integrated bioengineering modelling and protocols to effectively manage the incorporation of algae into wastewater treatment for resource recovery and biofuel production have received relatively less attention in the scientific literature. While some modelling efforts are carried out much of the research is still exploratory in scope and narrative in nature. The evidence evaluated here suggests that progress will require translation of the ever-expanding experimental data into the development of management systems based on applied process models. A shift in focus from nutrient removal to optimization of nutrient utilisation may be required. Advancement will also depend factors outside the strictly scientific activity; however, a focused system approach will be beneficial for the successful translation of current understanding into sustainable practice.

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### **References**

- [1] The Commissioners Appointed to Inquire and Report what Methods of Treating and Disposing of Sewage (including any liquid from any factory or manufacturing process) may be Properly Adopted. General Summary of Conclusions and Recommendations. Presented to both Houses of Parliament and of His Majesty, Published by His Majesty's Stationery Office, London UK, 1915.
- [2] Anonymous, A Review of Literature of 1967 on Wastewater and Water Pollution Control - Water Pollution - Eutrophication, *J Water Pollut Con F*, 40 (1968) 897-1222.
- [3] W.W. Eckenfelder, *Water quality engineering for practicing engineers*, Barnes & Noble, Inc., New York, NY, 1970.
- [4] D. Gleisberg, J. Kandler, H. Ulrich, P. Hartz, *Eutrophication and Wastewater Purification*, *Angew Chem Int Edit*, 15 (1976) 354-365.
- [5] H. Tamiya, *Mass Culture of Algae*, *Annual Review of Plant Physiology*, 8 (1957) 309-334.
- [6] J.R. Benemann, W.J. Oswald, *Integrating Wastewater-Treatment with Fuel Production Using Microalgae*, *J Electrochem Soc*, 126 (1979) C139-C139.
- [7] J. Sheehan, T. Dunahay, J. Benemann, P. Roessler, *Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae; Close-Out Report*, United States, 1998.
- [8] U.S.DOE, *National Algal Biofuels Technology Roadmap*, U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Biomass Program, 2010.
- [9] A. Kumar, S. Ergas, X. Yuan, A. Sahu, Q.O. Zhang, J. Dewulf, F.X. Malcata, H. van Langenhove, *Enhanced CO<sub>2</sub> fixation and biofuel production via microalgae: recent developments and future directions*, *Trends Biotechnol*, 28 (2010) 371-380.
- [10] F.G.A. Fernandez, C.V. Gonzalez-Lopez, J.M.F. Sevilla, E.M. Grima, *Conversion of CO<sub>2</sub> into biomass by microalgae: how realistic a contribution may it be to significant CO<sub>2</sub> removal?*, *Appl Microbiol Biot*, 96 (2012) 577-586.
- [11] J.C.M. Pires, M.C.M. Alvim-Ferraz, F.G. Martins, M. Simoes, *Carbon dioxide capture from flue gases using microalgae: Engineering aspects and biorefinery concept*, *Renew Sust Energ Rev*, 16 (2012) 3043-3053.
- [12] S.A. Razzak, M.M. Hossain, R.A. Lucky, A.S. Bassi, H. de Lasa, *Integrated CO<sub>2</sub> capture, wastewater treatment and biofuel production by microalgae culturing-A review*, *Renew Sust Energ Rev*, 27 (2013) 622-653.
- [13] J.B.K. Park, R.J. Craggs, A.N. Shilton, *Wastewater treatment high rate algal ponds for biofuel production*, *Bioresource Technol*, 102 (2011) 35-42.
- [14] C.M. Beal, A.S. Stillwell, C.W. King, S.M. Cohen, H. Berberoglu, R.P. Bhattarai, R.L. Connelly, M.E. Webber, R.E. Hebner, *Energy return on investment for algal biofuel production coupled with wastewater treatment*, *Water Environment Research*, 84 (2012) 692-710.
- [15] J.S. Burlew, V. Bush, *Algal culture from laboratory to pilot plant*, Washington, 1953.
- [16] J. Myers, *The growth of *Chlorella pyrenoidosa* under various culture conditions*, *Plant Physiology*, 19 (1944) 579-589.
- [17] G.G. Vinberg, *[Cultivation of green plankton algae on sewage]*, *Mikrobiologiya*, 33 (1964) 508-515.
- [18] C. Vilchez, I. Garbayo, M.V. Lobato, J.M. Vega, *Microalgae-mediated chemicals production and wastes removal*, *Enzyme Microb Tech*, 20 (1997) 562-572.
- [19] R. Munoz, B. Guieysse, *Algal-bacterial processes for the treatment of hazardous contaminants: A review*, *Water Res*, 40 (2006) 2799-2815.

- [20] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: A review, *Renew Sust Energ Rev*, 14 (2010) 217-232.
- [21] L. Brennan, P. Owende, Biofuels from microalgae-A review of technologies for production, processing, and extractions of biofuels and co-products, *Renew Sust Energ Rev*, 14 (2010) 557-577.
- [22] R. Rajkumar, Z. Yaakob, M.S. Takriff, Potential of the Micro and Macro Algae for Biofuel Production: A Brief Review, *Bioresources*, 9 (2014) 1606-1633.
- [23] S. Chen, Z. Chi, J.V. O'Fallon, Y. Zheng, M. Chakraborty, D.D. Laskar, System integration for producing microalgae as biofuel feedstock, *Biofuels*, 1 (2010) 889-910.
- [24] A. Dibenedetto, The potential of aquatic biomass for CO<sub>2</sub>-enhanced fixation and energy production, *Greenhouse Gases: Science and Technology*, 1 (2011) 58-71.
- [25] Y.M. Gong, M.L. Jiang, Biodiesel production with microalgae as feedstock: from strains to biodiesel, *Biotechnol Lett*, 33 (2011) 1269-1284.
- [26] N.K. Singh, D.W. Dhar, Microalgae as second generation biofuel. A review, *Agron Sustain Dev*, 31 (2011) 605-629.
- [27] A. Parmar, N.K. Singh, A. Pandey, E. Gnansounou, D. Madamwar, Cyanobacteria and microalgae: A positive prospect for biofuels, *Bioresource Technol*, 102 (2011) 10163-10172.
- [28] L. Christenson, R. Sims, Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts, *Biotechnol Adv*, 29 (2011) 686-702.
- [29] H.N. Chanakya, D.M. Mahapatra, S. Ravi, V.S. Chauhan, R. Abitha, Sustainability of large-scale algal biofuel production in India, *Journal of the Indian Institute of Science*, 92 (2012) 63-98.
- [30] D. Aitken, B. Antizar-Ladislao, Achieving a Green Solution: Limitations and Focus Points for Sustainable Algal Fuels, *Energies*, 5 (2012) 1613-1647.
- [31] M.K. Lam, K.T. Lee, Microalgae biofuels: A critical review of issues, problems and the way forward, *Biotechnol Adv*, 30 (2012) 673-690.
- [32] M.Y. Menetrez, An Overview of Algae Biofuel Production and Potential Environmental Impact, *Environ Sci Technol*, 46 (2012) 7073-7085.
- [33] H.M. Amaro, A.C. Macedo, F.X. Malcata, Microalgae: An alternative as sustainable source of biofuels?, *Energy*, 44 (2012) 158-166.
- [34] E.J. Olguin, Dual purpose microalgae-bacteria-based systems that treat wastewater and produce biodiesel and chemical products within a Biorefinery, *Biotechnol Adv*, 30 (2012) 1031-1046.
- [35] U.B. Singh, A.S. Ahluwalia, Microalgae: a promising tool for carbon sequestration, *Mitig Adapt Strat Gl*, 18 (2013) 73-95.
- [36] T. Cai, S.Y. Park, Y.B. Li, Nutrient recovery from wastewater streams by microalgae: Status and prospects, *Renew Sust Energ Rev*, 19 (2013) 360-369.
- [37] V. Makareviciene, V. Skorupskaite, V. Andruleviciute, Biodiesel fuel from microalgae-promising alternative fuel for the future: a review, *Rev Environ Sci Bio*, 12 (2013) 119-130.
- [38] J.C.M. Pires, M.C.M. Alvim-Ferraz, F.G. Martins, M. Simões, Wastewater treatment to enhance the economic viability of microalgae culture, *Environmental Science and Pollution Research*, 20 (2013) 5096-5105.
- [39] S.K. Prajapati, P. Kaushik, A. Malik, V.K. Vijay, Phycoremediation coupled production of algal biomass, harvesting and anaerobic digestion: Possibilities and challenges, *Biotechnol Adv*, 31 (2013) 1408-1425.

- [40] N.C. Bhatt, A. Panwar, T.S. Bisht, S. Tamta, Coupling of Algal Biofuel Production with Wastewater, *Sci World J*, (2014).
- [41] W.G. Zhou, P. Chen, M. Min, X.C. Ma, J.H. Wang, R. Griffith, F. Hussain, P. Peng, Q.L. Xie, Y. Li, J. Shi, J.Z. Meng, R. Ruan, Environment-enhancing algal biofuel production using wastewaters, *Renew Sust Energy Rev*, 36 (2014) 256-269.
- [42] K.F. Kamarudin, D.G. Tao, Z. Yaakob, M.S. Takriff, M.S.A. Rahaman, J. Salihon, A review on wastewater treatment and microalgal by-product production with a prospect of palm oil mill effluent (POME) utilization for algae, *Der Pharma Chemica*, 7 (2015) 73-89.
- [43] W. Klinthong, Y.H. Yang, C.H. Huang, C.S. Tan, A Review: Microalgae and Their Applications in CO<sub>2</sub> Capture and Renewable Energy, *Aerosol Air Qual Res*, 15 (2015) 712-742.
- [44] A. Mehrabadi, R. Craggs, M.M. Farid, Wastewater treatment high rate algal ponds (WWT-HRAP) for low-cost biofuel production, *Bioresource Technol*, 184 (2015) 202-214.
- [45] S.O. Gultom, B. Hu, Review of Microalgae Harvesting via Co-Pelletization with Filamentous Fungus, *Energies*, 6 (2013) 5921-5939.
- [46] M.L. Gerardo, S. Van Den Hende, H. Vervaeren, T. Coward, S.C. Skill, Harvesting of microalgae within a biorefinery approach: A review of the developments and case studies from pilot-plants, *Algal Res*, 11 (2015) 248-262.
- [47] J.U. Grobbelaar, Microalgal biomass production: challenges and realities, *Photosynth Res*, 106 (2010) 135-144.
- [48] S.K. Wang, A.R. Stiles, C. Guo, C.Z. Liu, Microalgae cultivation in photobioreactors: An overview of light characteristics, *Eng Life Sci*, 14 (2014) 550-559.
- [49] J.U. Grobbelaar, Algal Biotechnology: Real Opportunities for Africa, *Phycologia*, 44 (2005) 39-39.
- [50] S.R. Subashchandrabose, B. Ramakrishnan, M. Megharaj, K. Venkateswarlu, R. Naidu, Consortia of cyanobacteria/microalgae and bacteria: Biotechnological potential, *Biotechnol Adv*, 29 (2011) 896-907.
- [51] N.K. Sharma, S.P. Tiwari, K. Tripathi, A.K. Rai, Sustainability and cyanobacteria (blue-green algae): facts and challenges, *J Appl Phycol*, 23 (2011) 1059-1081.
- [52] J. Peccia, B. Haznedaroglu, J. Gutierrez, J.B. Zimmerman, Nitrogen supply is an important driver of sustainable microalgae biofuel production, *Trends Biotechnol*, 31 (2013) 134-138.
- [53] J.J. Tate, M.T. Gutierrez-Wing, K.A. Rusch, M.G. Benton, The Effects of Plant Growth Substances and Mixed Cultures on Growth and Metabolite Production of Green Algae *Chlorella* sp.: A Review, *J Plant Growth Regul*, 32 (2013) 417-428.
- [54] N. Rashid, M.S.U. Rehman, J.I. Han, Recycling and reuse of spent microalgal biomass for sustainable biofuels, *Biochem Eng J*, 75 (2013) 101-107.
- [55] A.J. Ward, D.M. Lewis, B. Green, Anaerobic digestion of algae biomass: A review, *Algal Res*, 5 (2014) 204-214.
- [56] J.H. Wang, H.Z. Yang, F. Wang, Mixotrophic Cultivation of Microalgae for Biodiesel Production: Status and Prospects, *Appl Biochem Biotech*, 172 (2014) 3307-3329.
- [57] J. Lowrey, M.S. Brooks, P.J. McGinn, Heterotrophic and mixotrophic cultivation of microalgae for biodiesel production in agricultural wastewaters and associated challenges-a critical review, *J Appl Phycol*, 27 (2015) 1485-1498.
- [58] S.Y. Chiu, C.Y. Kao, T.Y. Chen, Y.B. Chang, C.M. Kuo, C.S. Lin, Cultivation of microalgal *Chlorella* for biomass and lipid production using wastewater as nutrient resource, *Bioresource Technol*, 184 (2015) 179-189.

- [59] G. Markou, I. Angelidaki, D. Georgakakis, Microalgal carbohydrates: an overview of the factors influencing carbohydrates production, and of main bioconversion technologies for production of biofuels, *Appl Microbiol Biot*, 96 (2012) 631-645.
- [60] M. Kesaano, R.C. Sims, Algal biofilm based technology for wastewater treatment, *Algal Res*, 5 (2014) 231-240.
- [61] M. Gross, D. Jarboe, Z.Y. Wen, Biofilm-based algal cultivation systems, *Appl Microbiol Biot*, 99 (2015) 5781-5789.
- [62] P.J. Schnurr, D.G. Allen, Factors affecting algae biofilm growth and lipid production: A review, *Renew Sust Energ Rev*, 52 (2015) 418-429.
- [63] A.W.D. Larkum, I.L. Ross, O. Kruse, B. Hankamer, Selection, breeding and engineering of microalgae for bioenergy and biofuel production, *Trends Biotechnol*, 30 (2012) 198-205.
- [64] D.L. Sutherland, C. Howard-Williams, M.H. Turnbull, P.A. Broady, R.J. Craggs, Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production, *Bioresource Technol*, 184 (2015) 222-229.
- [65] D. Benson, K. Kerry, G. Malin, Algal biofuels: impact significance and implications for EU multi-level governance, *J Clean Prod*, 72 (2014) 4-13.
- [66] P.K. Usher, A.B. Ross, M.A. Camargo-Valero, A.S. Tomlin, W.F. Gale, An overview of the potential environmental impacts of large-scale microalgae cultivation, *Biofuels*, 5 (2014) 331-349.
- [67] G. Acien Fernandez, J.M. Fernandez, J.J. Magan, E. Molina, Production cost of a real microalgae production plant and strategies to reduce it, *Biotechnol Adv*, 30 (2012) 1344-1353.
- [68] S.H. Huo, R.J. Dong, Z.M. Wang, C.L. Pang, Z.H. Yuan, S.N. Zhu, L. Chen, Available Resources for Algal Biofuel Development in China, *Energies*, 4 (2011) 1321-1335.
- [69] G. Acien Fernandez, C.V. González-López, J.M. Fernández Sevilla, E. Molina Grima, Conversion of CO<sub>2</sub> into biomass by microalgae: How realistic a contribution may it be to significant CO<sub>2</sub> removal?, *Appl Microbiol Biot*, 96 (2012) 577-586.
- [70] V. Montemazzani, I.C. Duggan, I.D. Hogg, R.J. Craggs, A review of potential methods for zooplankton control in wastewater treatment High Rate Algal Ponds and algal production raceways, *Algal Res*, 11 (2015) 211-226.
- [71] N. Renuka, A. Sood, R. Prasanna, A.S. Ahluwalia, Phycoremediation of wastewaters: a synergistic approach using microalgae for bioremediation and biomass generation, *Int J Environ Sci Te*, 12 (2015) 1443-1460.
- [72] V.V. Unnithan, A. Unc, G.B. Smith, Mini-review: A priori considerations for bacteria-algae interactions in algal biofuel systems receiving municipal wastewaters, *Algal Res*, 4 (2014) 35-40.
- [73] S. Chinnasamy, A. Sood, N. Renuka, R. Prasanna, S.K. Ratha, S. Bhaskar, R. Rengasamy, D.M. Lewis, Ecobiological aspects of algae cultivation in wastewaters for recycling of nutrients and biofuel applications, *Biofuels*, 5 (2014) 141-158.
- [74] L.D. Zhu, Microalgal culture strategies for biofuel production: a review, *Biofuel Bioprod Bior*, 9 (2015) 801-814.
- [75] G.Y. Chen, L. Zhao, Y. Qi, Enhancing the productivity of microalgae cultivated in wastewater toward biofuel production: A critical review, *Appl Energ*, 137 (2015) 282-291.
- [76] N. Abdel-Raouf, A.A. Al-Homaidan, I.B.M. Ibraheem, Microalgae and wastewater treatment, *Saudi J Biol Sci*, 19 (2012).
- [77] P.M. Mambo, D.K. Westensee, B.M. Zuma, A.K. Cowan, The Belmont Valley integrated algae pond system in retrospect, *Water Sa*, 40 (2014) 385-393.

- [78] X.H. Zeng, X.Y. Guo, G.M. Su, M.K. Danquah, S.D. Zhang, Y.H. Lu, Y. Sun, L. Lin, Bioprocess considerations for microalgal-based wastewater treatment and biomass production, *Renew Sust Energ Rev*, 42 (2015) 1385-1392.
- [79] S. Judd, L.J.P. van den Broeke, M. Shurair, Y. Kuti, H. Znad, Algal remediation of CO<sub>2</sub> and nutrient discharges: A review, *Water Res*, 87 (2015) 356-366.
- [80] N. Brown, A. Shilton, Luxury uptake of phosphorus by microalgae in waste stabilisation ponds: current understanding and future direction, *Rev Environ Sci Bio*, 13 (2014) 321-328.
- [81] A.N. Shilton, N. Powell, B. Guieysse, Plant based phosphorus recovery from wastewater via algae and macrophytes, *Current Opinion in Biotechnology*, 23 (2012) 884-889.