

Studies on NO_x removal using *Dunaliella salina* algae in photobioreactors

Kethineni Chandrika¹, S. F. Choragudi², Krishna Kireeti Kakarla³, Kolluru Sumanth⁴ and Ch Devika⁵

¹Associate Professor, Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, AP, India-522502

^{2,3,4,5}Department of Biotechnology, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, AP, India-522502

ABSTRACT

The capability of an algal species to remove NO₂ and NO in the simulated flue gas was established using *Dunaliella salina* in Photobioreactors under two variants of NO_x sources. The concentrations studies were in the range between 25ppm to 150ppm. The diffusion of NO_x and subsequent reaction with water resulted in NO₃⁻ and NO₂⁻ in the growth medium. Algal growth by absorption of NO₃⁻ and NO₂⁻ created a nitrate gradient in the bulk medium resulting in NO_x uptake rates from the gas phase of up to 96%, leaving the unconsumed nitrogen of up to 7 mg-N/L in the growth medium. Algal species having an initial cell density of 2.8x10⁵ cells/mL grew to the cell density of 1.73x10⁷ cells/mL and dry weight of 262 mg/L. The Nitrogen content of cells varied from 3-6%. The treatment of NO_x in Photobioreactors was investigated with reference to the gas removal efficiency, cell growth and total nitrogen content in the biomass

KEY WORDS: *DUNALIELLA SALINA*, PHOTOBIOREACTORS, ALGAL GROWTH

INTRODUCTION

Disproportionate usage of fossil fuels has been considered as the source for manmade toxic emissions comprising carbon dioxide, sulfur dioxide, nitrogen oxides, volatile organic compounds and heavy metals (Mulhol-

land, 2008; Attilo et al., 2009). The by-products of fossil fuels have been identified as one of the major anthropogenic sources of this gas, contributing to global warming by the greenhouse effect. Therefore, it has become obligatory to reduce these toxic emissions before they are disposed into the environment. Nitric oxide (NO) and

ARTICLE INFORMATION:

Corresponding Authors: kkchandrika@kluniversity.in

Received 19th Oct, 2018

Accepted after revision 24th Dec, 2018

BBRC Print ISSN: 0974-6455

Online ISSN: 2321-4007 CODEN: USA BBRCBA

Thomson Reuters ISI ESC / Clarivate Analytics USA

Mono of Clarivate Analytics and Crossref Indexed

Journal Mono of CR

NAAS Journal Score 2018: 4.31 SJIF 2017: 4.196

© A Society of Science and Nature Publication, Bhopal India

2018. All rights reserved.

Online Contents Available at: <http://www.bbrc.in/>

DOI: 10.21786/bbrc/11.4/18

Nitrogen dioxide (NO₂) are the two main components that make up NO_x. These components are toxic and have various environmental hazards as per Environmental Protection Agency (Ronda, 2014). The nitrogen removal is 94.9 % in the ammonium form nitrogen group using biofilm (Yuxuan Zhu 2018).

There are several methods for treatment of NO_x. Selective catalytic reduction (SCR) is used, however it is more expensive when applied for large-scale power plants (Miller et al., 2010). Another way to treat NO_x from stationary sources is to use scrubbers to transfer the risk into an aqueous solution, which still must be treated or disposed of (Raja et al., 2007). Hence, to develop an economical and practical process to handle NO_x exists. Cultivation of an algae to take up dissolved NO_x from a scrubber as a nitrogen source, and oxygen only released as a by-product. This concept has been worked to estimate that, algal strains and conditions, algae can take up nitrogen from dissolved NO_x (Nagase et al., 1997). Toxic compounds present in the flue gas inhibit the growth rate. NO₂ has high solubility in water and therefore, reacts with water to form aqueous nitrates (NO₃) and nitrites (NO₂), some of the unaccounted-for nitrogen was lost due to volatilization of gaseous nitrogen species, (Kaitlyan 2018).

Aqueous nitrate and nitrites are used by the algae as a source of nitrogen for cell synthesis, (Mulholland & Lomas, 2008). The dissolved NO₂ and NO react to form dissolved nitrogen compounds which are available to ingest, biological conditions for the uptake of nitrate or nitrite by the algae, (Lee & Schwartz 1981). The nitrate or nitrite uptake by the algae reduce the soluble NO₂, thus increases the concentration gradient of NO₂ between liquid and the air. Thus, apparent solubility of NO₂ is proportional to the NO₂ gradient in the bulk medium, (Skalska, et al 2010). Nitrogen mono-oxide and sulfur dioxide can be removed by simultaneous absorption into aqueous mixed solutions of sulfite and [Fe II (edta)]H₂O]²⁻, ferrous ion coordinated to an anion of ethylene-diaminetetraacetic acid, (Tomasz et al 2016). A sequential process for the recovery and purification of multiple products was used on a mixture of algal biomass comprised of *Spirulina platensis* and *Dunaliella salina* (Kethineni 2017).

Dunaliella salina is a green algae known to withstand high salinity and accumulate carotenes. The nitrate requirement for algae is more for accumulation of biomass than for accumulation of carotene. Nitrates in the range of 1- 10mM is suitable for algal growth (Tafreshi et al., 2009). Harter et al, (2012) performed a mass balance for nitrogen from NO_x for *Dunaliella* cultures in a column reactor. In a lab scale trial under simulated flue gas the results indicate that with an inflow of

150 µg N/L day NO_x along with CO₂ the algae could be able to maintain a net influx of 0.52.73 µg N L⁻¹ d⁻¹ which amounts to 35% NO_x removal. Nagase et al, (1997) studied the removal rate of nitric oxide by *Dunaliella tertio-lacta* supplied in the range between 25-500ppm. At an inlet concentration of 500ppm NO in addition to CO₂, it was shown to remove 110 µmoles per hour at a flow rate of 150mL/min. Also, it was shown that within a range of 100 to 400mL/min gas flow rate, a maximum of 60% of the NO was removed. These results suggest that *D. salina* is a potential algal species for NO_x removal. The ability of the green algae, *Chlorella* to acclimate to high level of NO_x and the potential usage of *Chlorella* strains in biological NO_x removal (DeNO_x) from industrial flue gases, (Tianpei and Gang Xu 2016). To understand the NO_x removal process and to increase its range of applicability. The use of microalgae for simultaneous removal of CO₂, SO_x and NO_x from flue gas is an environmentally benign process, (Hong-Wei Yen et al, 2016), 75% decrease of the nitrogen concentration in the medium, with respect to the optimal values for growth, increased the lipid fractions of algal species, (Attilio Converti et al 2009).

It is very important to undertake biological NO_x fixation. Therefore, in this work, two individual experiments were conducted to productively remove the NO_x from simulated flue gas with varying NO_x loading rates by estimating the optimal growth parameters. Different NO_x concentrations were supplied to each photobioreactor inoculated with *Dunaliella salina*. NO_x removal efficiency and algal growth were determined in each experiment.

MATERIAL AND METHODS

DUNALIELLA CULTURE

All three reactors were inoculated with 600 mL of pure *Dunaliella salina* (SAG:42.88) grown in *Dunaliella* medium (=Dun) at 25°C and a pH of 7.0. The inoculum was grown to a 1 x10⁷ cells/mL, with an initial cell density of 2.8x10⁵ cells/mL

GROWTH MEDIUM

The modified *Dunaliella* growth medium was used for inoculum and algae growth experimentation. All the nitrogen uptake by the algal cells was provided through inlet simulated gas. *Dunaliella salina* was grown in modified *Dunaliella* medium. A nitrogen free stock solution was prepared with K₂HPO₄, 0.1 g/ 100 mL. 20 mL of this nutrient was mixed with 30 mL of the soil extract and 930 mL artificial seawater to make a liter solution. The growth medium was given in Table 1.

Component	Stock sol. (g/100mL)	Nutrient concentration (mL)
KOH	0.1	20
Soil extract	30	-
Artificial seawater	930	-

Operating conditions of the reactor

Case 1: pure NO₂ feed source:

NO₂ gas diluted with ambient air was used as the simulated flue gas for the first run. As the boiling point of NO₂ is approximately 20°C at atmospheric pressure; NO₂ was initially released as a liquid in the tubing. NO₂ was blended with 3Lpm of air to get NO_x concentrations of 100ppm, 200ppm, 350 ppm in photobioreactors 1a, 1b, and 1c respectively. Experimental conditions were given in Table 2.

Setting	Reactor 1a	Reactor 1b	Reactor 1c
Inlet NO _x (ppm)	100	200	350
Inlet Gas Flow Rate (Lpm)	3	3	3
pH	7-8	7-8	7-8
Temperature (°C)	20	20	20

The simulated gas entered each photobioreactor through a sparger, pH was maintained in the range of 7.0 to 8.0 using CO₂. The carbon dioxide feed was monitored by separate valves to each reactor which was controlled based on the pH in the reactor. NO_x removal rates were monitored for four days. The reactors were illuminated with three 1 m long fluorescent white lights emitting, a total of 2700 Klux.

Case 2: Gas feed source calibration:

For the case 2, the reactors were inoculated before to the start of NO_x loading and left for two days during which, only ambient air was supplied to the system. NO_x was given from NO₂ calibration gas cylinders compris-

Setting	Reactor 2a	Reactor 2b	Reactor 2c
Inlet NO _x (ppm)	25	50	120
Inlet Gas Flow Rate (Lpm)	3	3	3
Influent CO ₂ (g) (ppm)	400	400	400
pH	7-8	7-8	7-8
Temperature (°C)	20	20	20

ing 5000 ppm and 9000 ppm NO₂ concentration. Thus, in this case, the need for the liquid NO₂ trap was ignored as the gas mixture was already in a vapor state. Thus, pumping calibration gas achieved steady inlet concentrations. Experimental conditions were given in table 3. The calibrated NO₂ is blended using air for the required NO_x concentrations. The calibrated gases were supplied to the reactor at 3 Lpm, having NO₂ concentrations of 25 ppm, 50 ppm, 120 ppm respectively. The concentration range was chosen to resemble real-time power plant NO_x concentrations. Two aquarium stones of 12cm were used to diffuse the gas into the reactor. CO₂ was supplied in the system, at a concentration of 400ppm until the pH remains 7 However when the pH is below 7, the pH 7 was maintained using 1.0 g/L solution of sodium bicarbonate (NaHCO₃). The system was run for six days after loading of NO_x. Influent and effluent concentrations of NO and NO₂ in gas samples were measured using an analyser, (Testo 350-S/-XL, USA).

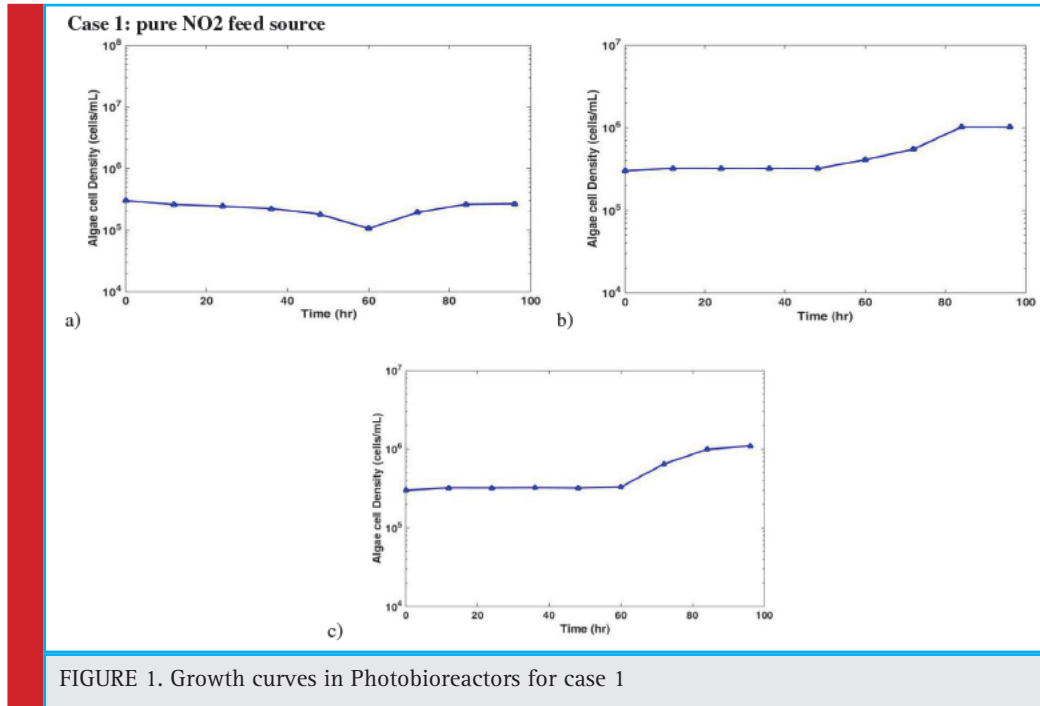
RESULTS AND DISCUSSION

NO_x REMOVAL EFFICIENCY

Case 1: pure NO₂ feed source

In this case, the reactors were fed with pure NO₂ which is delivered as a liquid at room temperature and then the collected vapors were diluted with air. Using pure NO₂ gas, actual average inlet concentrations for reactors 1a, 1b, and 1c were 108 ppm, 35ppm, 70 ppm respectively (Table 4). Fig. 1 shows the *Dunaliella* growth curve in reactors 1a, 1b, and 1c during 90-hour run.

Reactor	Ratio (NO:NO ₂)	Inlet NO _x (g) Conc (ppm)	Outlet NO _x (g) Conc (ppm)	Average removal Conc (%)
1a	0.47	108±55	47±33	49
1b	0.15	35±16	15±12	51
1c	0.35	70±32	8±7	81
2a	0.038	27±6	11±4	59
2b	0.035	57±9	2±8	96
2c	0.043	126±12	7±11	95



All three reactors began with 2.8×10^5 cells/ml. As shown in Fig. 1, the culture in reactor 1a began exhaustive during the first 24 hours, therefore, the cells never attained a density greater than the initial. The maximum cell densities, for 1b, 1c were 1.46×10^6 cells/mL and 1.48×10^6 cells/mL respectively. Table 3 presents the NO_x removal data for the reactors under case 1. Inlet and out-

let NO_x in Table 3 is the summation of measured NO and NO₂ concentrations in the inlet and outlet streams. The efficiency of NO_x removal by the algal system is based on total nitrogen through the reactor system and not on any particular NO_x component. Therefore, removal percent of total NO_x is only considered in the analysis. Reactor 1a obtained an average NO_x removal of 49%,

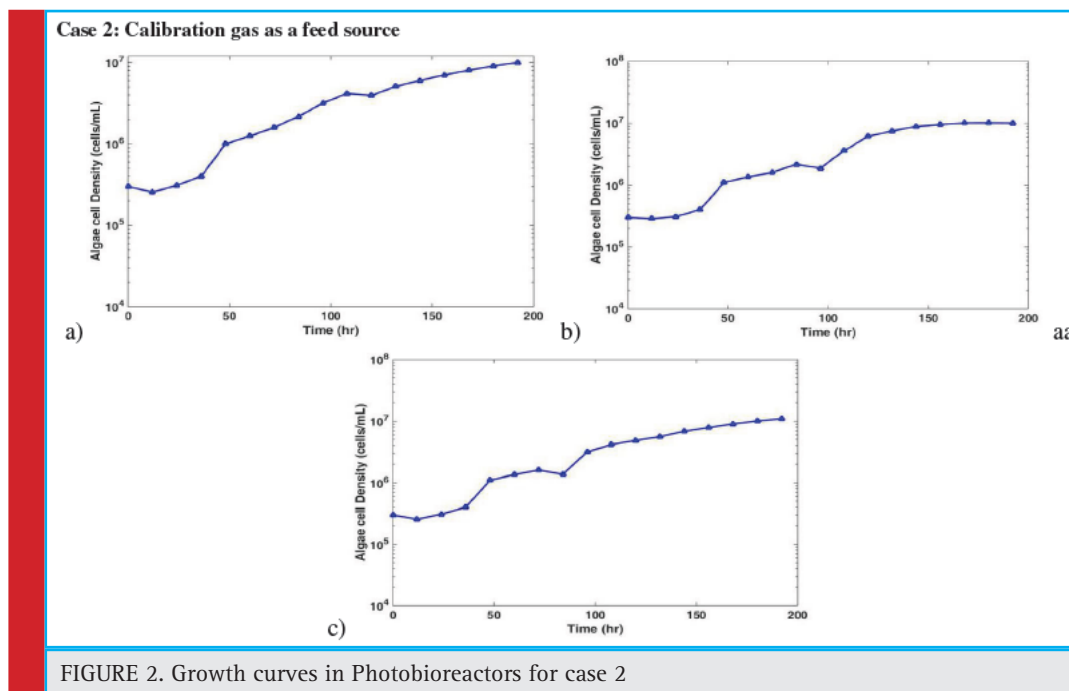


Photo-bioreactor	TSS Initial (mg/L)	TSS Final (mg/L)
2a	6.0	210
2b	5.7	243
2c	6.3	222

Reactor 1b achieved an average 51% NOx removal, and Reactor 1c has removed an average of 81% of inlet NOx. The average NOx removal efficiency for the first 60 h was 39%, but the average removal for the last 25 hours was 52%. As the algal culture was declining, removal of NOx could be associated with the dissolution of NO₂ into the medium.

Reactors 1b and 1c presented moderate growth following an extended lag phase, as shown in the figure 1. Algae in reactors 1b, 1c has not truly experienced log phase, and the run was ended after 90 hours.

Case 2: Calibration gas as a feed source

As shown in Fig. 2 all three reactors began with a cell concentration of 2.8x10⁵ cells/mL for three reactors 2a, 2b, and 2c. Maximum cell densities were 1.24x10⁷ cells/mL, 1.46x10⁷ cells/mL and 1.72x10⁷ cells/mL respectively. To overcome some of the problems that appeared from using pure NO₂ gas in case 1, NO₂ calibration gases of 5000ppm and 10000 ppm NO₂ were used to supply NOx for case 2. This allowed for accurate NOx loading concentrations. Actual NOx Loading rates for case 2 were 27 ppm, 57 ppm and 126 ppm for reactors 2a, 2b, and 2c respectively.

The data in Table 4 presents the removal of NOx for the reactors (case 2). Inlet and outlet NOx in Table 4 is the summation of measured NO and NO₂ concentrations in the inlet and outlet streams. Reactor 2a got an average NOx removal of 59% reactor 2b got an average 96%, and reactor 2c was able to remove an average of 95% of inlet NOx. The lag phase was reduced to less than 24 hours due to delay in NOx loading, and log phase was attained between 24 and 48 hours. NOx loading started

	Reactor 2a	Reactor 2b	Reactor 2c
NO _x Input	890	1740	3450
NO _x Output	450	60	90
N Consumed from NO _x gas phase	440	1680	3360
Initial N in growth medium	23	22	23
Final NO ₃ ⁻	27	14	48
Final NO ₂ ⁻	74	13	163
Net Accumulation of NO ₃ ⁻ + NO ₂ ⁻	78	5	188
Initial Organic N	0	0	0
Final Organic N	310	750	1140
N Accumulated in Algal Cells	310	750	1140
Total N Accumulated	388	755	1228
Mass Balance (% NO _x uptake accounted for)	88%	45%	36%

at 48 hours, and three reactors showed continued growth, but with a significant decline in growth rate (Fig. 2).

Total suspended solids (TSS) for initial and final samples were taken to quantify algal growth and to estimate the nitrogen content of the cells. The results were summarized in the Table 5. Total suspended solid results show that 37 fold average mass growth was accomplished over the 190-hour run. For nitrate and nitrite concentrations, liquid samples from case 2 were analyzed, and the results were shown in Fig.3. Nitrate was completely drained in all three reactors before NOx was loaded into the system at 49 hours. Nitrogen source available for algal growth only after that point was from dissolved NO₂. Initial and final total organic nitrogen content of the algal cultures was used to estimate the uptake of nitrogen by algae and to determine the nitrogen content of the cells. These analyses summarized in the table: the cells in reactors 2a, 2b, and 2c were found to contain 6.2%, 4.1%, and 7.9% nitrogen respectively.

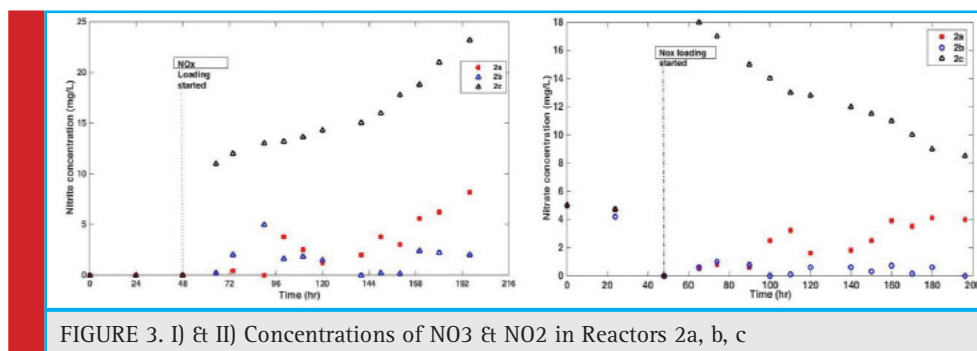


FIGURE 3. I) & II) Concentrations of NO₃ & NO₂ in Reactors 2a, b, c

A mass balance of nitrogen was conducted to assess the effect of NO_x through the system. In reactor 2a, the mass balance alleged for 88% of the observed NO_x removal, as slightly more nitrogen was found in the cells and growth medium than entering the system.

For reactors 2b and 2c, only 45% and 36% respectively of the nitrogen that entered the system was found in the cells and growth medium. NO_x feed stream: Loading NO_x at a particular concentration tested to be very difficult for the first case, as can see by more standard deviations in the table 6. In the first case, NO_x was given from a pure NO₂ cylinder, and vapors from this liquid were pumped using peristaltic pumps into the reactors. To overcome large deviations in concentration, case 2 was operated using calibration gases. Data analysis considered only in case 2 because of the NO_x feed was not consistent during case 1. Nitrogen mass balance data summarized in Table 6. The only difference in the three reactors in case 2 was the loading rates of NO_x.

NO_x removal: In case 2, NO_x removal rates are 59%, 95%, 96% for reactors 2a, 2b, and 2c respectively. Only 59% NO_x removal was achieved in reactor 2a, which had the lowest NO_x loading rate. NO_x loading rates were different in three reactors. Aqueous nitrate and nitrite concentrations in the reactors are shown in Figure 3.

Cell growth: The average specific growth rate for the first 2 days in reactors 2a, 2b, and 2c was 0.03, 0.06, 0.12 respectively, the average specific growth rates for the same period in reactors 1a, 1b were both negative, the growth curves of case 2 presented in Fig. 3, after loading NO_x, growth rates started to decline in all three reactors and never achieved the value as before NO_x loading, and this declining growth is proportional to the NO_x concentration entering the system. Approximately 48 hours of NO_x loading, the inlet concentrations of NO_x do not affect algal growth. Algae took nearly 48 hours to adopt new nitrogen source.

Nitrate /Nitrite: The algae was able to take nitrate in solution before the loading of into the reactors. After 48 hours Nitrogen source is available only from NO_x. The steady fall in NO₃⁻ concentrations and NO₂⁻ accumulation in reactor 2c suggests that algae preferred NO₃⁻ as its nitrogen source over NO₂⁻.

CONCLUSION

The primary purpose of this study was to test the hypothesis that *Dunaliella* can grow on nitrogen from dissolved NO₂ as its only nitrogen source for cell synthesis. *Dunaliella* grew used only nitrate /nitrite generated by the NO_x dissolution for cell synthesis, reaching

a maximum cell density of 1.75x10⁷cell/ml. A reactor with lower NO₂ loading concentrations resulted in lower NO_x removal rates, for this reactor, nitrate was not accumulated as efficiently as a similar with grated NO_x loading. In case 2: cell growth of mass between 1850mg/L to 198mg/L. Nitrogen was removed from gaseous NO_x at a rate of 0.06-0.45 mg N/mg cell growth. Assuming a 700 MW natural gas fired power plant can produce up to 1,70,000m³/h of flue gas with approx. 50ppm NO_x concentrations, growth of a minimum 110 kg algal cell/h would be required to treat this stream.

REFERENCES

- Attilo Converti et al (2009) Effect of temperature and nitrogen concentration on the growth and lipid content of *Nanochloropsis lata* and *Chlorella vulgaris* for biodiesel production Chemical Engineering and Processing Vol.48 1146-1151
- Harter H J (2013) Carbon and nitrogen mass balance during flue gas treatment with *Dunaliella salina* cultures, Journal of Applied Phycology, Volume 2 : 359-368
- Hong-Wei Yen (2015) CO₂, NO_x and SO_x removal from flue gas via microalgae cultivation, Biotechnology Journal Vol. 10: 890-899.
- Kaitlyan D (2018) The fate of nitrogen through algal treatment of landfill leachate, Algal Research, Volume 30: 50-58
- Kethineni, C. (2017) Development of Sequential Processes for Multiple Product Recovery from Microalgae, Industrial Biotechnology Vol 14 No 2:94-99
- Lee, Y. N., & Schwartz, S. E. (1981) Reaction kinetics of nitrogen dioxide with liquid water at low partial pressure. The Journal of Physical Chemistry. Vol 85 No7: 840-848
- Miller, J. S., & Ledakowicz, S. (2010) Trends in NO_x abatement: A review. Science of the Total Environment, Vol 408 No 19:3976-3989.
- Mulholland, M. R., & Lomas, M. W. (2008) Nitrogen Uptake and Assimilation. In D. G. Capone, D. A. Brown, M. R. Mulholland, & E. J. Carpenter (Eds.), Nitrogen in the marine environment Amsterdam: Elsevier Academic Press. (2nd Ed.: 303-384).
- Nagase, H. (1997) Characteristics of biological NO_x removal from flue gas in a *Dunaliella tertiolecta* culture system. Journal of Fermentation and Bioengineering, Vol. 83 No 5: 461-465
- Raja R S. Hemaiswarya, R. Rengasamy (2007) Exploitation of *Dunaliella* for β-carotene production. Applied Microbiology & Biotechnology. Vol 74 No 3: 517-523
- Ronda JI (2014) A growth inhibitory model with SO_x influenced effective growth rate for estimation of algal biomass concentration under flue gas atmosphere, Bioresource Technology, Volume 152: 283-291
- Skalska K (2010), Trends in NO_x abatement. Implementation of stringent regulations of NO_x emission requires the development of new technologies for NO_x removal from exhaust gases. Science of The Total Environment Vol. 408: 3976-3989

Kethineni Chandrika *et al.*

Tianpei Li and Gang Xu (2016). The acclimation of *Chlorella* to high-level nitrite for potential application in biological NO_x removal from industrial flue gases. *Journal of Plant Physiology* Vol. 195: 73-79.

Tomasz T & Hidehiro Kumazawa (2016) Reactivity of nano-size zinc powder in the aqueous solution of (FeIII(Edta)

(H₂O) *Science of The Total Environment* Vol. 628-629: 870-881

Yuxuan Zhu (2018), Biological activities and nitrogen and phosphorus removal during the *Anabaena flos-aquae* biofilm growth using different nutrient form, *Bioresource Technology*, Volume 251: 7-12