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NO_x Enriched Flue Gas Fixation for Biomass Production of *Chlorella Vulgaris* Buitenzorg

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Cultivation of *Chlorella vulgaris* Buitenzorg in a pilot scale of bubble column photo bioreactor using simulated NO_x enriched flue gas concluded that presence of N_2O as simulated NO_x pollution (0.02%) in blowing bubbled air and CO_2 is not so significant, compare to control experiment that was designed by absence of N_2O (around 20% decreased). Meanwhile, presence of N_2O tends a less significantly decreasing of μ - specific growth rate and q_{CO2} – specific CO_2 transferred rate. It is around 30% decreased in both of μ and q_{CO2}. Then, cultivation by presence of NO_x in blowing simulated flue gas could drastically decreased intracellular carotene and lipid content and become increase to level near to both of pigment and lipid content in control experiment. Furthermore, cultivation by presence of NO_x in blowing simulated flue gas also could drastically exchange intracellular fatty acid content and it become dominated by 16:0 species. Finally, refreshing cellular growth product with re-cultivation by blowing fresh air, could be restored the fatty acid content nearly to beginning microbial fatty acid content. It was happened cause of converting hexadecanoate species to octadecanoate species and it was shown that oleate (18:1) was dominating species.

Keywords: *Chlorella vulgaris* Buitenzorg, NO_x, photo bioreactor, scale up

INTRODUCTION

Global warming has become one of the most serious environment problems. The main cause of this is because of the increasing of CO_2 level in the atmosphere. In recent years, many attempts have been done to reduce the quantity of CO_2 in the atmosphere. Studies on photosynthesis, CO_2 fixation and utilization of micro algae biomass has been carried out. Similar to another *Chlorella* strain, *Chlorella vulgaris* Buitenzorg is known widely of its high valued potential substances such as chlorophyll, CGF, carotene, and protein, and it can be used as potential biomass albeit the function of CO_2 fixation [Wirosaputro, 2002; Wijanarko et al, 2005; Wijanarko et al, 2006a; Wijanarko et al, 2006b] and also possible content long chain un–saturated fatty acid potencies biodiesel as a renewable fuel stock.

Previous research objectives are to increase CO_2 fixation and the amount of biomass *Chlorella vulgaris* Buitenzorg with various types of illumination (e.g. continuous illumination, cycle illumination, and alteration illumination) at a single and also multiple photo bioreactor [Hirata et al, 1996; Jenkins et al, 1989;Morita et al 1999; Wijanarko et al, 2004; Wijanarko and Ohtaguchi, 2004; Wijanarko et al, 2006a; Wijanarko et al, 2006b, Wijanarko et al, 2005; Wijanarko et al 2007a, Wijanarko et al, 2007b]. This research uses a large flat surface photo bioreactors as a part of scale up design for large scale biomass production by using combination flue gas utilization as carbon source.

MATERIALS AND METHODS

Chlorella vulgaris Buitenzorg is taken from Depok Fresh Water Fishery Research Center that was grown in Benneck medium. This strain grows in 18.0 dm^3 of culture medium in bubble column photo bioreactor that have sizing of (38.5 cm x 10 cm x 60 cm). An operation condition was defined as following.

Temperature (T) was set at 29.0 °C (302 K), Pressure (P) was set at ambient pressure (1 atm.; 101 kPa), Light intensity (I) was set at 3.0 Klx, superficial gas velocity (U_G) was set at 15.7 m/h and CO_2 concentration (y_{CO2i}) in blown bubble air was set around 5.0% and as additional purposes was also enriched by 0.02% N_2O as an existence side product NO_x species in simulated flue gas. Before cultivation, this strain was grown with pre-culture condition that was set by blowing bubble fresh air with U_G 1.0 vvm with similar operation condition. These photo bioreactors are illuminated by 4 (four) lamps [*Philips Halogen* lamp 20W/12V/50Hz]. Experimental apparatus used in the experiment is shown on Figure 1.

Culture biomass content (OD_{600} method) was measured at 600 nm using UV-Vis Spectrophotometer (Labo-Med Inc.), Lipid content is analysis by Bligh-Dyer Method [Manirakizal, 2001]; extracted fatty acid content is analyzed using GCMS; elemental analysis is done by XRD and CHNS analyzer; CO_2 inlet and outlet is measured using TCD Gas Chromatography; Chlorophyl a and carotene content is assayed and calculated by pigment assay procedure [Richmond, 2004]; and finally, ☒ - specific growth rate and q_{CO2} – specific CO_2 transferred rate is calculated with correlation that was notified in previous result [Wijanarko et al, 2006a; Wijanarko et al, 2006b].

RESULT AND DISCUSSION

For industrial application purposes, additional step using simulated flue gas was modified with mixing of 0.02% (v/v) N_2O and 5.0% (v/v) CO_2 content in blowing bubbled air. This simulation experiment aimed for utilization of the flue gas of Gas Electrical Power Generator or Gas Firing Boiler which contained combusted side product NO_x below 1.0 % in flue gas before mixing with atmospheric air to make concentration around 5.0% and blowing into bubble column for biomass cultivation. Experimental result of biomass concentration X was shown in Figure 2, concluded that presence of N_2O as simulated NO_x pollution (0.02%) in blowing bubbled air and CO_2 (2.67g/dm^3) is not so significant compare to control experiment (3.42 g/dm^3) that was designed by absence of N_2O (around 20% decreased). This simulated bubbling flue gas cultivation result was done during 188 h cultivation period and at least was quite similar to control experiment result.

This phenomenon was caused by internal microbial system of *Chlorella*, which was predicted similar to other microalgae such as *Anabaena, Nostoc* and *Chlorella* that have several cellular mechanism pathway to proceeded rarely content NO_x to make a by-

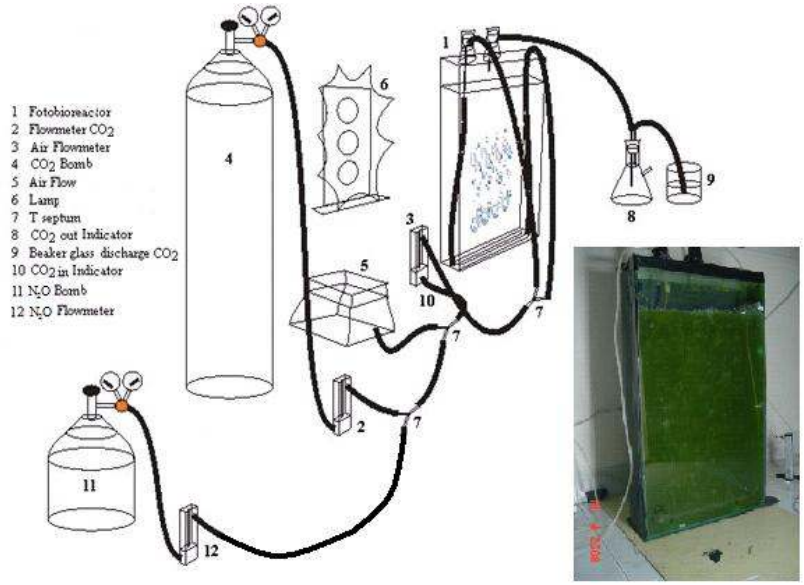

Figure 1. Experimental Apparatus

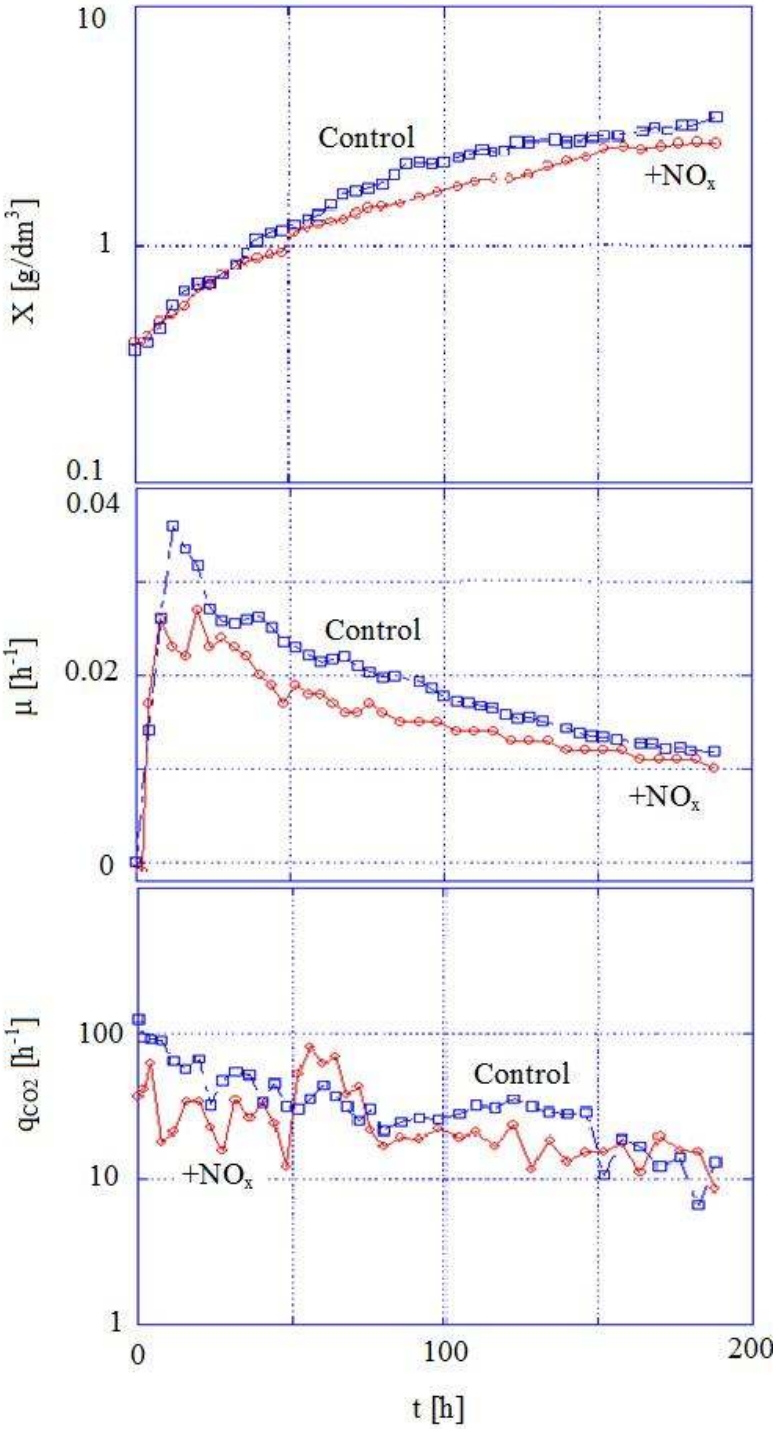

Figure 2*. The results of Biomass Production of *Ch. v.* Buitenzorg (X) by the existence of N_2O as simulated NO_x pollution (0.02%) in bubbled mixing air and CO_2 (+NO_x); and absence of N_2O (Control)
*) All data shown in Figure 3 was the average value of 3 (three) measured replicates with standard deviation (**δ**) of 10%

product nitrite or nitrate molecules that was furthermore transformed to amino acid and other organic substances by cellular TCA cycle and advanced mechanism pathway [Lee et al, 2002; Wijanarko and Ohtaguchi, 2005]. Meanwhile, presence of N_2O tends a less significantly decreasing of μ - specific growth rate (0.28 h^{-1}) and q_{CO2} – specific CO_2 transferred

Table 1. Elemental analysis result of dry biomass of *Ch. v.* Buitenzorg

Element Species	% (w/w)
Carbon	43.1
Hydrogen	23.7
Nitrogen	10.2
Oxygen	18.5
Phosphor	4.5

Table 2. Intracellular carotene, chlorophyl-a as an antioxidant agent and lipid content of *Ch. v.* Buitenzorg by blowing: 5% CO_2 enriched bubbling air (control); 5% CO_2 enriched bubbling air and also adding 0.02% N_2O as an existence side product species in flue gas (NO_x); Reuse pre-culture to micro algae after cultivate by control (R_{PC}.Control); Reuse pre-culture to micro algae after cultivate by NOx ($R_{PC}.NO_x$).

Species (Range Content)[Becker, 2004]	Control	NO_x	R_{PC}.Control	$R_{PC}.NO_x$
Carotene [3.3 – 11.2]; (ppm)	9.42	5.60	11.6	8.71
Chlorophyl [7.0 – 27.0]; (ppm)	17.9	16.8	43.3	22.8
Lipid [14.0 – 22.0]; (%)	18.3	11.8	18.8	14.3

Table 3. Intracellular Fatty Acid Composition of *Ch.v.* Buitenzorg by blowing: 5% CO_2 enriched bubbling air (control); 5% CO_2 enriched bubbling air and also adding 0.02% N_2O as an existence side product species in flue gas (NO_x); Reuse pre-culture to micro algae after cultivate by control (R_{PC}.Control); Reuse pre-culture to micro algae after cultivate by NOx ($R_{PC}.NO_x$).

Fatty Acid (%)	Control	$+NO_x$	R_{PC}.Control	$R_{PC}.NO_x$
16:0	24.7	43.4	27.4	27.3
16:1	5.82	ND	ND	1.14
16:2	3.45	1.23	3.35	0.163
18:0	ND	ND	ND	0.112
18:1	22.5	22.8	24.7	37.8
18:2	18.3	12.4	20.9	17.0
18:3	25.1	20.74	23.37	16.95

ND, Not Detected

rate (27.6 h^{-1}). It is around 30% decreased in both of μ and q_{CO2}.

Table 1 shown elemental analysis result that was a combination result of XRD analyzer to get exact oxygen content and CHNS analyzer to find other elements content. From above result, it could be concluded that biomass formula is: $CH_{3.3}N_{0.203}O_{0.322}P_{0.041}$. Further, reaction of microbial growth could be simplified as

$$CH_{3.3}N_{0.203}O_{0.322}P_{0.041} + 1.11\ H_2O + HCO_3^- + 0.041\ H_2PO_4^- + 0.203\ NO_3^- \rightarrow 2CH_{3.3}N_{0.203}O_{0.322}P_{0.041} + 4.56\ O_2$$

For re-increasing internal pigment, lipid and also fatty acid content, biomass product is refreshed by reuse pre-culture method that was not increasing biomass product but a little bit could change whole internal composition of biomass product. Table 2 and 3 were shown whole biomass internal content result.

Table 2 shown a analytical result of internal carotene, chlorophyll a, as an antioxidant agent and lipid content of this strain after cultivating by blowing bubbled air that was enriched by CO_2 5% and also adding N_2O as an existence side product species in flue gas. Above table was also shown result content after reuse pre-culture both of these biomass products. This result, except antioxidant content after reuse pre-culture to micro algae after cultivate by control, It was in the range of another microalgae strain such *Chlorella, Spirulina, Dunaliella* and so on [Becker, 2004]. Cultivation by presence of NO_x in blowing simulated flue gas could drastically decreased intracellular carotene and lipid content and become increase to level near to both of pigment and lipid content in control experiment.

Table 3 shown a analytical result of internal fatty acid composition of this strain similar to

above table explanation that was analyzed after cultivate by blowing bubbling air that was enriched by CO_2 5% and also adding N_2O as an existence side product species in flue gas. This table was also shown fatty acid composition after redoing pre-culture both of these biomass products. Similar to result on another eukaryotic strains such palm oil, canola oil and so on [Becker, 2004; ,Nasikin et al, 2009; Nasikin et al, 2008; Idem, !997; Twaiq et al, 1999], the fatty acid composition of this strain is dominated with hexadecanoate and octadecanoate species while another species such tetradecanoic (palmitate) that was know exist in prokaryotic strain is not detected. Cultivation by presence of NO_x in blowing simulated flue gas could drastically exchange intracellular fatty acid content become dominated by 16:0 species. It was could be understood by decreasing of intracellular lipid content significantly from 18.3% to be 11.8% cause of un-complete intracellular anabolism processing by presence of oxidative species NO_x in simulated flue gas. Refreshing cellular growth product with re-cultivation by blowing fresh air (reuse pre-culture) could be restored the fatty acid content nearly to beginning microbial fatty acid content. It was happened cause of converting hexadecanoate species to octadecanoate species and shown oleate species (18:1) was dominant. This result was also understood by small increasing in intracellular lipid content (14.3%) after reuse pre-culture. It was notified this refreshing mode of both + NO_x and control cultivation un-significantly change lipid content.

Base on above concluded result, for pharmacy seeding purpose, it will be designed to combine cultivation that was done such in control experiment and after that it is continued by reuse pre-culturing process, for increasing both of pigment and lipid content with un-significantly change in intercellular fatty acid content. In the other hand, to solve global warming issues, blowing bubbled mixing air with flue gas as CO_2 source from Gas Electrical Power Plant or Gas Firing Boiler in industrial processing that was also contain NO_x SO_x as combustion side product, is possible to apply for commercial bio-energy production purposes. After biomass sizing reduction processing, it is need to combine with extraction processing for separating lipid content from other hydrophilic substance such starch, cellulose and other carbohydrates, protein, free amino acid, pigment and so on. It was known in eukaryotic microalgae such *Chlorella, Dunaliella* and *Spirulina*, starch content is around 30 – 40% w/w [Richmond, 2004; Wijanarko and Ohtaguchi, 2005]. Further it is suggested to produce with both of fermentation processing using *Sacharamyces sake* and *Sacharomyces cereviceae* for producing ethanol from by product starch and cellulose, and also trans-esterification processing for producing biodiesel from by product lipid.

CONCLUSION

Cultivation of *Ch. v.* Buitenzorg for large scale biomass production purposes in flat type bubble column photo bioreactor using simulated enriched flue gas, consist of N_2O as simulated NO_x pollution (0.02%) in blowing bubbled air and CO_2, is not so significant compare to control experiment (3.42g/dm^3) that was designed by absence of N_2O (2.67g/dm^3 and around 20% decreased). Meanwhile, presence of N_2O tend a less significantly decreasing of μ - specific growth rate (0.28 h^{-1}) and q_{CO2} – specific CO_2 transferred rate (27.6 h^{-1}). It is around 30% decreased in both of μ and q_{CO2}. Then, cultivation by presence of NO_x in blowing simulated flue gas could drastically decreased intracellular carotene and lipid content and become increase to level near to both of pigment and lipid content in control experiment. Furthermore, cultivation by presence of NO_x in blowing simulated flue gas also could drastically exchange intracellular fatty acid content and it become dominated by 16:0 species. It was could be understood by decreasing of intracellular lipid content significantly from 18.3% to be 11.8% cause of un-complete intracellular anabolism processing by presence of oxidative species NO_x in simulated flue gas. Finally, refreshing cellular growth product with re-cultivation by blowing fresh air could be restored the fatty acid content nearly to beginning microbial fatty acid content that was converting hexadecanoate species to octadecanoate and shown oleate species (18:1) was dominant. This result was also understood by small increasing in

intracellular lipid content (14.3%) after this reuse pre-culture.

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REFERENCES

Becker W (2004) Microalgae in human and animal nutrition, Handbook of Microalgae Culture, Blackwell, Oxford, 312-351 [Book article]

Hirata, S., M. Taya and S. Tone (1996), *Characterization of Chlorella cell cultures in batch and continuous operations under a photoautotrophic condition*, Journal Chemical Engineering of Japan, 29, 953-959 [Journal article]

Idem, R.O., S.P.R. Katikaneni, N.N. Bakhsi (1997), Catalytic Conversion of Canola oil to Fuels and Chemicals: Role of Catalyst Acidity, Basicity, and Shape Selectivity on Product Distribution. Fuel Process. Tech. 51, 101 – 125 [Journal article]

Jenkins C. L. D., R. T. Furbank and M. D. Hatch (1989), *Mechanism of C4 photosynthesis, a model describing the inorganic carbon pool in bundle sheat cells, Plant Physiology*, 91, 1372-1381 [Journal article]

Lee, J.S, Kim D.K, Lee J.P., Park S.C., Cho H.S., Kim S.W. (2002), *Effect of SO_2 and NO on growth of Chlorella sp. K-R 1*. Bio-resource Technology, 82, 1 -4

Manirakizal P., A.Covaci, P. Schepens (2001), *Comparative Study on Total Lipid Determinationusing Soxhlet, Roese Gottlieb, Bligh Dyer, and Modified Bligh Dyer Extraction Method*, 14, 93 – 100 [Journal article]

Morita E., T. Abe, M. Tzusuki, S. Fujiwara, N. Sato, A. Hirata, K. Sonoike and H. Nozaki (1999), *Role of pyrenoids in the CO_2-contrating mechanism: comparative morphology, physiology and molecular phylogenetic analysis of closely related strains of Chlamydomonas and Chloromonas (Volvocales),* Planta, 208, 365-372 [Journal article]

Nasikin M., B. H. Susanto, M. A. Hirsaman and A. Wijanarko (2009), *Biogasoline from Palm Oil by Simultaneous Cracking and Hydrogenation Reaction over NiMo/Zeolite Catalyst*, World Applied Sciences Journal, 5, 74-79 [Journal article]

Nasikin M., A. Wijanarko, B. H. Susanto, *Bensin-bio yang disintesa dari minyak nabati triolein berikut metode sintesanya* (P00200800789 – 11 Desember 2008) [Patent]

Richmond A. [Ed.] (2004) *Handbook of Microalgal Culture: Biotechnology and Applied Phycology*, 40 – 54 [Journal article]

Twaiq, F.A., N.A.M. Zabidi, S. Bhatia (1999), *Catalytic Conversion of Palm Oil to Hydrocarbons: Performance of Various Zeolite Catalysts*. Industrial Engineering and Chemical Resources, 38, 3230 – 3232 [Journal article]

Wijanarko A., Dianursanti, A. B. Witarto and Roekmijati Widaningroem Soemantojo (2004), *Effect of photoperidicity on CO_2 fixation by Chlorella vulgaris* Buitenzorg *in bubble column photobioreactor for food supplement production*, Makara, 8, 35-44 [Journal article]

Wijanarko A. and K. Ohtaguchi (2004), *Carbon dioxide removal and biomass production by Anabaena cylindrica IAM M1 using reactor in series"*, Studies in Surface Science and Catalysis, 153, 461-468 [Journal article]

Wijanarko A., S.M.K. Andika, Dianursanti, A.B. Witarto, K. Asami, K. Ohtaguchi and R.W. Soemantojo, *Alteration of Light illumination during microbial growth, an enhancement effort of carbon dioxide fixation and biomass production by Chlorella vulgaris* Buitenzorg, 8th International Conference on Carbon Dioxide Utilization, Oslo, Norway, June 20-23, 2005 (OC 06 pp. 48-49) [Conference paper]

Wijanarko A. and K. Ohtaguchi (2005), "*Double-ended helix of blue green alga 16S ribosomal RNA, a suitable environmental temperature subsequence*", ACGC

Chemical Research Communications, 19, 16-20 [Journal article]

Wijanarko A., Dianursanti, Heidi, R W. Soemantojo and K. Ohtaguchi (2006a), *"Effect of Light Illumination alteration on Chlorella vulgaris* Buitenzorg*'s* CO_2 *fixation in bubble column photobioreactor",* International Journal for Algae, 8,. 53-60 [Journal article]

Wijanarko A., Dianursanti, M. Gozan, S. M. K. Andika, P. Widiastuti, H. Hermansyah, A. B. Witarto, K. Asami, R. W. Soemantojo, K. Ohtaguchi, S. K. Song (2006b), *"Enhancement of carbon dioxide fixation by alteration of illumination during Chlorella vulgaris* Buitenzorg*'s growth"*, Biotechnology and Bioprocess Engineering, 11, 484-488 [Journal article]

Wijanarko A., Dianursanti, A.Y. Sendjaya, H. Hermansyah, A.B. Witarto, M. Gozan, B.T. Sofyan, K.Asami, K. Ohtaguchi, R. W. Soemantojo and S. K. Song (2007a), *Enhanced Chlorella vulgaris* Buitenzorg *Growth by Photon Flux Density Alteration in Serial Photobioreactors*, ASEAN Journal of Chemical Engineering, 7, 89-101 [Journal article]

Wijanarko A., Dianursanti, M. A. Sujarwo and M. Gozan, *An enhancement effort of Biomass Production by Chlorella vulgaris* Buitenzorg*'s in serial configuration of Bubble Column Photo Bioreactor*, First Indonesia-Korea Biotechnology Symposium, Jakarta, Indonesia, January 30th, 2007b (OP 06 pp. 1-6) [Conference paper]

Wirosaputro, S. (2002), *Chlorella Untuk Kesehatan Global*, Gadjah Mada University Press, Yogyakarta [Book]