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Low Cost Carbon and Nitrogen Sources for Higher Microalgal Biomass and Lipid Production Using Agricultural Wastes

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ABSTRACT

The aim of this study was to evaluate the hydrolysates from crop residues as low cost carbon and nitrogen source for algal cultivation. The enzymatic hydrolysates of sweet sorghum (SSH) and rice straw (RSH) were used for heterotrophic cultivation of *Chlorella vulgaris* and *Scenedesmus obliquus*. Sugar concentration of 36.5 and 30.3 g L⁻¹ were obtained in SSH and RSH, whereas, the nitrogen content was 10.6 and 19.5 mg L⁻¹. Basal media with glucose was used as control and hydrolysates medium was used alone and in combination to determine its efficiency on microalgal biomass and lipid production. Maximum biomass was achieved in combined hydrolysates medium in *C. vulgaris* (4.8 g L⁻¹) followed by *S. obliquus* (4.3 g L⁻¹). Total lipid content of *Chlorella* was ranged from 11.26-29.36 and 15.43-27.24% in *Scenedesmus*. The qualitative analysis of fatty acids showed very high values of stearic acid (28.41 and 31.01%) and palmitic acid (23.54 and 26.21%) in both microalgae. This study could establish that the hydrolysates from sweet sorghum stem and rice straw can be used as growth medium for microalgal cultivation and opens new possibilities of exploiting crop residues for industrial applications.

Key words: Biofuel, microalgae, biomass, lipid production, *Chlorella*, *Scenedesmus*

INTRODUCTION

Microalgae cultivation using large quantities of fertilizers raises questions on its environmental impact (Sialve *et al.*, 2009; Lardon *et al.*, 2009). Finding cheap and efficient resources is important to replace fertilizers usage in cultivating microalgae. Though microalgae represent a promising candidate for biofuel production, the cost of fermentation substrate reduces its commercialization. For example, the cost of glucose accounted for 80% of the total medium cost (Li *et al.*, 2007). Typically, lower price of fermentation substrate and raw materials reduce the cost of algal cultivation for biomass and lipid production. The increasing demand of biodiesel makes in search of alternative cheap sources and agricultural wastes are notable. Cellulosic biomasses are abundant renewable resources for the production of biofuel (Himmel *et al.*, 2007) and are commonly used as carbon/energy source (Lee and Fan, 1983). Agricultural residues would help to ease world energy crisis, if they could be converted to energy efficiently. Microalgae are capable of transforming organic carbon sources to lipid as intracellular products (Heredia-Arroyo *et al.*, 2011; Miao and Wu, 2006). Use of hydrolysates from agricultural residues as cultural medium for microalgal growth is one of the cost effective methods, as many species of microalgae take up organic compounds from the medium. Organic carbon sources like starch hydrolysates from Jerusalem asrichoke (Cheng *et al.*, 2009a), sweet sorghum (Gao *et al.*, 2010), cassava (Lu *et al.*, 2010), waste molasses

(Yan *et al.*, 2011) and rice straw (Li *et al.*, 2011) were utilized to cultivate microalgae as cost effective approach to displace glucose. Enhanced algal lipid production using hydrolysates from oil crop biomass residues was reported earlier (Wang *et al.*, 2013).

The major criteria for the selection of agricultural wastes to be used in algal cultivation are the availability, cost and nutrient content. Sweet sorghum is cultivated in most of parts of the world and the fresh stem yield is more than 45 t ha⁻¹ per year (Gnansounou *et al.*, 2005). Sweet sorghum stem is rich in sugars, mainly with sucrose, fructose and glucose (Billa *et al.*, 1997), which serves as ideal culture media. Rice straw making up of 50% weight of the crop is one of the largest lignocellulose resources and its hydrolysate mainly contains glucose, xylose and arabinose (Parajo *et al.*, 1998). In the present study, the application of sweet sorghum and rice straw for biomass and lipid production by microalgae was investigated under heterotrophic conditions. The efficiency of biomass and lipid production from hydrolysates of sweet sorghum and rice straw were compared alone and in combination.

MATERIALS AND METHODS

Isolation and purification of the microalgae: Waste water was collected from Bangalore Water Sewerage and Supply Board (BWSSB), Bengaluru (13°04'N, 77°58'E), India and poured into a closed 250 mL bottle and exposed in sunlight for 3 weeks. The upper layer of the water was inoculated into BG11 medium enriched agar plates containing 200 µg mL⁻¹ ampicillin. The plates were incubated at 25±2°C under cool white fluorescent light (40 µmol photons m⁻² sec⁻¹; 15 h light/9 h dark) until algal growth was detected. Single green colour colonies were inoculated into BG11 medium and the algal growth was measured spectrophotometrically. Among the colonies, cultures shown maximum specific growth rate were selected and identified as *Chlorella vulgaris* and *Scenedesmus obliquus* according to Andersen (2005) and Round (1973).

Enzymatic hydrolysis: The agro wastes used in this study were sorghum stalks and rice straw. The residues were dried in room temperature and in hot air oven for 48 h followed by milling into fine powder (0.5 mm). Cellulase (Sigma) was used for the hydrolysis of sorghum stems and rice straw. After the addition of cellulase, the mixture was vortexed well and then maintained at 55°C. After hydrolysis, the reducing sugar content in the enzymatic hydrolysates was determined by HPLC.

Culture conditions: Microalgae cultivations were performed in 500 mL conical flasks under heterotrophic conditions. In order to compare the effects of hydrolysates from sweet sorghum stem and rice straw on heterotrophic growth and lipid production of microalgae, 4 kinds of media including basal media (Xiong *et al.*, 2008), Sweet Sorghum Hydrolysate (SSH) medium, Rice Straw Hydrolysate (RSH) medium and combination of SSH and RSH medium were prepared (Table 1). Basal medium supplemented with 30 g L⁻¹ glucose was used as control and the final concentrations of reducing sugar and total nitrogen in hydrolysate medium were adjusted to 30 g and 10 mg L⁻¹, respectively. Logarithmic cells of *Chlorella* and *Scenedesmus* were inoculated into culture medium and cultivated at 28±2°C with continuous shaking at 250 rpm.

Measurement of algal growth: The cell growth of microalgae was determined by measuring the Optical Density (OD) at 680 nm using a spectrophotometer. The specific growth rate (µ) was obtained on the exponential logarithmic growth phase by plotting the concentrations of biomass versus time using the Eq. 1 (Wang *et al.*, 2012):

Table 1: Composition of culture media

Ingredients	Basal medium	SSH	RSH	SSH+RSH
KH ₂ PO ₄ (g L ⁻¹)	0.7	0.7	0.7	0.7
K ₂ HPO ₄ (g L ⁻¹)	0.3	0.3	0.3	0.3
MgSO ₄ .6H ₂ O (g L ⁻¹)	0.3	0.3	0.3	0.3
FeSO ₄ .7H ₂ O (g L ⁻¹)	3.0	3.0	3.0	3.0
Vitamin B ₁ (mg L ⁻¹)	0.01	0.01	0.01	0.01
A ₅ trace mineral solution (mg L ⁻¹)	1.0	1.0	1.0	1.0
Glycine (g L ⁻¹)	0.1	0.1	0.1	0.1
Yeast extract (g L ⁻¹)	2.0	-	-	-
Glucose (g L ⁻¹)	30.0	-	-	-
Reducing sugar (g L ⁻¹)	-	30.0	30.0	30.0
Total nitrogen (mg L ⁻¹)	-	10.0	10.0	20.0

$$\mu = \frac{\ln (C_{t2} / C_{t1})}{t_2 - t_1} \quad (1)$$

where, C_t are cell concentration at different time points (t₁ and t₂), respectively.

Dry weight determination: A 20 mL algae solution was centrifuged and the pellet was dried at 70°C in hot air oven until getting a constant weight. The biomass productivity (P, mg L⁻¹ day⁻¹) was calculated as shown in Eq. 2 (Ho *et al.*, 2012):

$$P = \frac{\Delta X}{\Delta t} \quad (2)$$

where, ΔX is the variation of biomass concentration (mg L⁻¹) within a cultivation time Δt (day).

Lipid extraction and estimation: Algal lipids were extracted according to the method of Folch *et al.* (1957). Briefly, the cells were centrifuged at 10000 rpm for 10 min and the pellet was homogenized with chloroform-methanol (2:1 v/v) solution. The sample was centrifuged and to the supernatant, 0.73% NaCl water was added to produce a final solvent system of 2:1:0.8 chloroform: methanol: water (v/v/v). The mixture was centrifuged and the lower organic phase was collected followed by evaporation under a steam of nitrogen. Palmitic acid was used a standard and the lipid content was determined from the standard curve at 350 nm. The eventual lipid yield was determined in both lipid content (%) and biomass yield (g L⁻¹) (Yan *et al.*, 2011) as shown in Eq. 3:

$$\text{Lipid yield (g L}^{-1}\text{)} = \text{lipid content (\%)} \times \text{biomass yield (g L}^{-1}\text{)} \quad (3)$$

Fatty acid methyl ester preparation and analysis: The Fatty Acid Methyl Esters (FAME) were converted from lipids and free fatty acids according to protocol of (Lepage and Roy, 1984). Algal cultures were centrifuged and 0.1 g of pellet was homogenized with 1.5 mL of acetyl chloride and methanol (20:1, v/v) in reaction vessels. Subsequently, 1 mL of hexane was added to the mixture and heated to 100°C for 1 h for derivatization. The mixture was cooled added with 1 mL of distilled water and the organic phase was separated by centrifugation and dried with anhydrous sodium sulphate. The extracts were filtered and FAME was analysed on GC-MS.

Statistical analysis: All results are expressed as Mean±Standard Deviation (SD) values. One-way Analysis of Variance (ANOVA) was used to evaluate the differences among the treatments.

RESULTS AND DISCUSSION

Enzymatic hydrolysates of crop residues: To obtain available carbon source from crop residues, cellulase was used and the enzyme hydrolysates mainly contained glucose, fructose, sucrose and arabinose (Table 2). Glucose showed the highest content and the maximum total sugar concentration (36.5 g L^{-1}) was obtained in Sweet Sorghum Hydrolysate (SSH). However, the total nitrogen content was higher in rice straw hydrolysate (19.5 mg L^{-1}). The result indicates that sweet sorghum was a good source of carbon and rice straw was rich in nitrogen content.

Effect of hydrolysates on microalgal growth: Algal biomass yield from hydrolysates medium were higher than that of basal media at 30 g L^{-1} sugar concentration. The dry cell weight of heterotrophic *Chlorella* and *Scenedesmus* with different media was shown in Fig. 1 and 2. At 20th day of cultivation, dry cell weight of *Chlorella* was 1.41, 1.24, 1.26 and 2.10 g L^{-1} in basal media, SSH, RSH and SSH+RSH media, respectively. Whereas, it was 1.44, 1.18, 1.30 and 2.09 g L^{-1} for *Scenedesmus* under the cultivating conditions. The cell dry weight of microalgae cultured in individual hydrolysate medium showed no significant differences but it was higher in combined hydrolysates medium (SSH+RSH).

Nitrogen affects the biomass growth and lipid productivity of microalgae (Griffiths and Harrison, 2009). Yeast extract is one of the most suitable nitrogen sources for microalgal cultivation (Xiong *et al.*, 2008) and the hydrolysate medium was prepared with a total nitrogen content of

Table 2: Composition of hydrolysates from sweet sorghum and rice straw

Composition	SSH	RSH
Glucose (g L^{-1})	19.25 ± 0.20	16.96 ± 0.11
Fructose (g L^{-1})	9.36 ± 0.01	7.41 ± 0.05
Sucrose (g L^{-1})	6.67 ± 0.14	4.87 ± 0.22
Arabinose (g L^{-1})	1.22 ± 0.07	1.10 ± 0.10
Total sugar (g L^{-1})	36.50 ± 0.44	30.34 ± 0.49
pH	4.70 ± 0.01	4.20 ± 0.01
Total nitrogen (mg L^{-1})	10.60 ± 0.10	19.50 ± 0.21

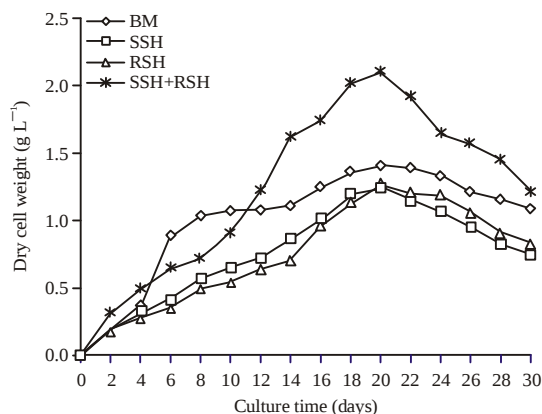


Fig. 1: Time course profile of cell growth curve in terms of dry cell weight of *Chlorella vulgaris*

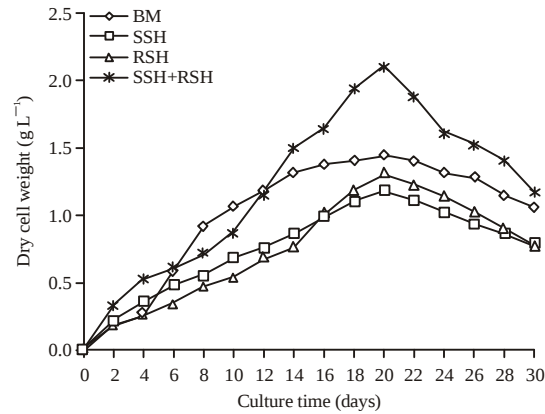


Fig. 2: Time course profile of cell growth curve in terms of dry cell weight of *Scenedesmus obliquus*

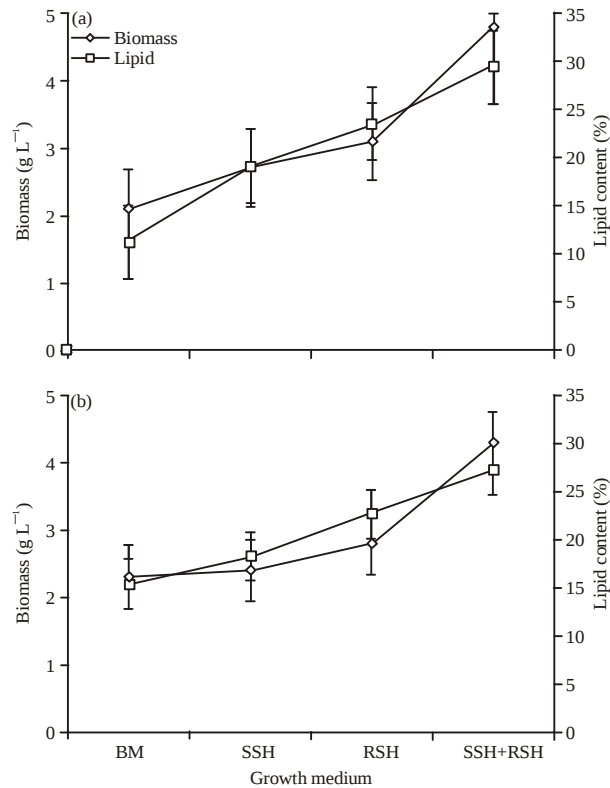


Fig. 3(a-b): Effect of culture media on biomass concentration and lipid content in (a) *C. vulgaris* and (b) *S. obliquus*

10 mg L⁻¹ in this study to determine the alternate nitrogen sources to promote algal growth and lipid production. In RSH medium, higher biomass was achieved (3.1 and 2.8 g L⁻¹) than SSH medium (2.7 and 2.4 g L⁻¹). However, the maximum biomass (4.8 and 4.3 g L⁻¹) was obtained when the medium was supplemented with both SSH and RSH. Although exogenous nitrogen source was not added into hydrolysate medium, maximum biomass reached 4.8 g L⁻¹ indicating the source of nitrogen from crop residues (Fig. 3a and b).

Effect of combined hydrolysates medium on biomass and lipid accumulation: It is important to determine the proportion of TAG in the total lipid content of microalgae cultured under heterotrophic conditions. Total lipid content of *Chlorella* ranged from 11.26-29.36% and 15.43-27.24% in *Scenedesmus* where, highest lipid content was obtained in combined hydrolysates medium (Table 3). The lipid yields of microalgae were determined and found increased in hydrolysates medium than basal medium. *Chlorella* has recorded 54.8% increase in lipid yield and 48.6% increase was observed in *Scenedesmus* cultivated in combination of SSH and RSH hydrolysates (Fig. 3a and b). In particular, cultures with total sugar concentration of 30 g L⁻¹ and 20 mg L⁻¹ of nitrogen content reached maximum biomass of 4.8 g L⁻¹ and fairly high lipid content (27-29%). These results showed that biomass and lipid productivity always change in parallel and relatively high lipid content could be achieved with the expense of algal growth.

Effect of combined hydrolysates medium on fatty acid composition: The lipids extracted from microalgae were converted to fatty acid methyl ester and their compositions were summarized in Table 4. Lipids preferred to accumulate more in hydrolysate medium rather than basal medium. The qualitative analysis of fatty acids showed very high values of stearic acid (28.41 and 31.01%) and palmitic acid (23.54 and 26.21%) in both microalgae. Other abundant fatty acids found were oleic, behenic, arachidic and myristic acid. Previous studies have shown that the quantity and quality of lipids within the cell may vary, based on changes in growth conditions (Illman *et al.*, 2000; Liu *et al.*, 2008). It was observed that hydrolysates medium influenced the lipid content of microalgae in which reason could be the presence of additional organics like fructose and arabinose.

Table 3: Growth rate, biomass and lipid productivity of *C. vulgaris* and *S. obliquus* in culture media

Agro wastes and microalgae	Maximum specific growth rate ($\mu_{\max}$ day ⁻¹)	Biomass productivity (mg L ⁻¹ day ⁻¹)	Lipid yield (g L ⁻¹)
BM			
<i>C. vulgaris</i>	0.23±0.27	47.57±0.01	0.37±0.41
<i>S. obliquus</i>	0.19±0.61	48.16±0.16	0.39±0.42
SSH			
<i>C. vulgaris</i>	0.21±0.03	61.34±0.11	0.47±0.02
<i>S. obliquus</i>	0.17±0.06	58.47±0.09	0.43±0.24
RSH			
<i>C. vulgaris</i>	0.25±0.10	69.74±0.20	0.59±0.06
<i>S. obliquus</i>	0.23±0.04	64.11±0.14	0.56±0.11
SSH+RSH			
<i>C. vulgaris</i>	0.34±0.13	103.20±0.25	0.82±0.13
<i>S. obliquus</i>	0.30±0.07	99.66±0.18	0.76±0.22

Table 4: Fatty acid composition produced in culture medium

	Basal media		SSH		RSH		SSH+RSH	
	<i>C. vulgaris</i>	<i>S. obliquus</i>	<i>C. vulgaris</i>	<i>S. obliquus</i>	<i>C. vulgaris</i>	<i>S. obliquus</i>	<i>C. vulgaris</i>	<i>S. obliquus</i>
Fame	------(%)-----							
Caprylic acid (C8:0)	0.39	0.47	0.16	0.09	0.62	0.57	0.93	0.84
Lauric acid (C12:0)	-	0.28	0.67	0.84	1.14	1.08	1.49	1.21
Myristic acid (C14:0)	2.07	1.81	2.38	1.94	3.67	2.93	11.37	9.68
Pentadecylic acid (C15:0)	1.26	0.95	1.37	1.24	2.06	1.92	3.19	2.89
Palmitic acid (C16:0)	14.69	16.37	15.30	17.04	16.05	18.36	23.54	26.21
Palmitoleic acid (C16:1)	-	0.85	-	1.17	-	1.81	0.37	2.06
Heptadecanoic acid (C17:0)	2.09	-	1.94	0.22	3.48	1.34	4.15	2.05
Stearic acid (C18:0)	16.37	18.96	17.14	16.28	22.38	23.54	28.41	31.01
Oleic acid (C18:1)	11.32	12.37	10.27	10.95	13.09	14.28	16.48	19.11
Linoleic acid (C18:2)	-	-	-	-	-	-	0.21	0.14
Arachidic acid (C20:0)	8.36	11.27	7.36	9.28	7.31	9.57	8.04	10.12
Behenic acid (C22:0)	10.24	11.87	6.96	7.21	7.32	9.07	8.45	9.37
Lignoceric acid (C24:0)	0.37	0.22	0.95	0.74	2.17	3.04	6.34	5.87

Higher levels of palmitic (C16:0) and stearic (C18:0) acid production from hydrolysates medium reveals the use of crop residues in microalgal lipid production, as it has been reported that carbon chain length of C16-C18 is important to produce suitable biodiesel (Miao and Wu, 2006).

Growth medium provides necessary nutrient sources for algal growth and under heterotrophic conditions, the cost of growth medium is high, therefore economic considerations demand much cheaper and easily available resources. A large amount of valuable compounds are present in crop residues after harvesting, which could be used as nutrient sources. Utilizing lignocellulosic biomass offers the possibility of renewable source of carbon and nitrogen that can be used to cultivate microalgae. Sorghum stalk and rice straw are the residues after harvesting and nutrients derived from them are potential to be low cost media for algal cultivation. Heterotrophic microalgae are capable of converting organic carbon sources to intracellular oil that could be used to produce biodiesel efficiently (Li *et al.*, 2007). Algal growth and lipid content are affected by the carbon source, nitrogen content and C/N in the medium (Cheng *et al.*, 2009b). The growth rate, cell density and productivity of microalgae were higher in heterotrophic cultures than phototrophic culture (Zheng *et al.*, 2012). Pretreatment of lignocellulosic biomass results in substantial breakdown and release of reducing sugars and other nutrients. Wheat bran treated with fungal extract was used as an efficient medium for the mixotrophic and heterotrophic growth of algal species (El-Sheekh *et al.*, 2012). In this study, cellulase was used to breakdown the lignocelluloses structure and the enzyme hydrolysates were used for microalgal cultivation. The results revealed that hydrolysates medium used alone and in combination influenced the algal biomass and lipid productivity than the basal medium. It was noted that combination of hydrolysates from sorghum stalk and rice straw proved cheap source of carbon and nitrogen for higher biomass and lipid production. This study could establish that the hydrolysates from agricultural wastes, can be used for microalgal cultivation and opens new possibilities of exploiting crop residues for industrial applications.

CONCLUSION

Utilization of crop waste materials for algal biomass and lipid production is a promising approach to meet the increasing energy needs as a substitute for fossil fuels. This work provides a novel strategy using sweet sorghum stem and rice straw as a potential carbon and nitrogen source in microalgal cultivation for biodiesel production. There is certainly the scope of enhancing the biomass and lipid yield by process optimization and the process with optimized cultivation conditions can then be used for scaling up to the pilot scale.

REFERENCES

- Andersen, R.A., 2005. Algal Culturing Techniques. Academic Press, USA., ISBN: 9780120884261, Pages: 578.
- Billa, E., D.P. Koullas, B. Monties and E.G. Koukios, 1997. Structure and composition of sweet sorghum stalk components. *Ind. Crops Prod.*, 6: 297-302.
- Cheng, Y., W. Zhou, C. Gao, K. Lan, Y. Gaw and Q. Wu, 2009a. Biodiesel production from Jerusalem artichoke (*Helianthus tuberosus* L.) tuber by heterotrophic microalgae *Chlorella protothecoides*. *J. Chem. Technol. Biotechnol.*, 84: 777-781.
- Cheng, Y., Y. Lu, C. Gao and Q. Wu, 2009b. Alga-based biodiesel production and optimization using sugar cane as the feedstock. *Energy Fuels*, 23: 4166-4173.
- El-Sheekh, M.M., M.Y. Bedaiwy, M.E. Osman and M.M. Ismail, 2012. Mixotrophic and heterotrophic growth of some microalgae using extract of fungal-treated wheat bran. *Int. J. Recycl. Org. Waste Agric.*, 1: 12.

- Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.
- Gao, C., Y. Zhai, Y. Ding and Q. Wu, 2010. Application of sweet sorghum for biodiesel production by heterotrophic microalga *Chlorella protothecoides*. *Applied Energy*, 87: 756-761.
- Gnansounou, E., A. Dauriata and C.E. Wyman, 2005. Refining sweet sorghum to ethanol and sugar: Economic trade-offs in the context of North China. *Bioresour. Technol.*, 96: 985-1002.
- Griffiths, M.J. and S.T.L. Harrison, 2009. Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *J. Applied Phycol.*, 21: 493-507.
- Heredia-Arroyo, T., W. Wei, R. Ruan and B. Hu, 2011. Mixotrophic cultivation of *Chlorella vulgaris* and its potential application for the oil accumulation from non-sugar materials. *Biomass Bioenergy*, 35: 2245-2253.
- Himmel, M.E, S.Y. Ding, D.K. Johnson, W.S. Adney, M.R. Nimlos, J.W. Brady and T.D. Foust, 2007. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. *Science*, 315: 804-807.
- Ho, S.H., C.Y. Chen and J.S. Chang, 2012. Effect of light intensity and nitrogen starvation on CO₂ fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresour. Technol.*, 113: 244-252.
- Illman, A.M., A.H. Scragg and S.W. Shales, 2000. Increase in *Chlorella* strains calorific values when grown in low nitrogen medium. *Enzyme Microb. Technol.*, 27: 631-635.
- Lardon, L., A. Helias, B. Sialve, J.P. Steyer and O. Bernard, 2009. Life-cycle assessment of biodiesel production from microalgae. *Environ. Sci. Technol.*, 43: 6475-6481.
- Lee, Y.H. and L.T. Fan, 1983. Kinetic studies of enzymatic hydrolysis of insoluble cellulose: (II). Analysis of extended hydrolysis times. *Biotechnol. Bioeng.*, 25: 939-966.
- Lepage, G. and C.C. Roy, 1984. Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J. Lipid Res.*, 25: 1391-1396.
- Li, X., H. Xu and Q. Wu, 2007. Large-scale biodiesel production from microalga *Chlorella protothecoides* through heterotrophic cultivation in bioreactors. *Biotechnol. Bioeng.*, 98: 764-771.
- Li, P., X. Miao, R. Li and J. Zhong, 2011. *In situ* biodiesel production from fast-growing and high oil content *Chlorella pyrenoidosa* in rice straw hydrolysate. *J. Biomed. Biotechnol.* 10.1155/2011/141207
- Liu, Z.Y., G.C. Wang and B.C. Zhou, 2008. Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresour. Technol.*, 99: 4717-4722.
- Lu, Y., Y. Zhai, M. Liu and Q. Wu, 2010. Biodiesel production from algal oil using cassava (*Manihot esculenta* Crantz) as feedstock. *J. Applied Phycol.*, 22: 573-578.
- Miao, X. and Q.Y. Wu, 2006. Biodiesel production from heterotrophic microalgal oil. *Bioresour. Technol.*, 97: 841-846.
- Parajo, J.C., H. Dominguez and J. Dominguez, 1998. Biotechnological production of xylitol. Part 3: Operation in culture media made from lignocellulose hydrolysates. *Bioresour. Technol.*, 66: 25-40.
- Round, F.E., 1973. *The Biology of the Algae*. 2nd Edn., Edward Arnold Publishers, London, UK., Pages: 23.
- Sialve, B., N. Bernet and O. Bernard, 2009. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. *Biotechnol. Adv.*, 27: 409-416.

- Wang, Y., T. Chen and S. Qin, 2012. Heterotrophic cultivation of *Chlorella kessleri* for fatty acids production by carbon and nitrogen supplements. *Biomass Bioenergy*, 47: 402-409.
- Wang, Z., X. Ma, W. Zhou, M. Min and Y. Cheng *et al.*, 2013. Oil crop biomass residue-based media for enhanced algal lipid production. *Applied Biochem. Biotechnol.*, 171: 689-689.
- Xiong, W., X. Li, J. Xiang and Q. Wu, 2008. High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production. *Applied Microbiol. Biotechnol.*, 78: 29-36.
- Yan, D., Y. Lu, Y.F. Chen and Q. Wu, 2011. Waste molasses alone displaces glucose-based medium for microalgal fermentation towards cost-saving biodiesel production. *Bioresour. Technol.*, 102: 6487-6493.
- Zheng, Y., Z. Chi, B. Luckner and A. Chen, 2012. Two-stage heterotrophic and phototrophic culture strategy for algal biomass and lipid production. *Bioresour. Technol.*, 103: 484-488.