International Journal of Technical Research and Applications e-ISSN: 2320-8163,
www.ijtra.com Special Issue 32 (September, 2015), PP. 49-55

ENERGY AND REVENUE CREATION FROM THE ANAEROBIC DIGESTION OF CHLORELLA VULGARIS CULTIVATED IN LIQUOR FROM CHEMICALLY TREATED SEWAGE SLUDGE.

Okoro-Shekwaga Cynthia Kusin[1], Nigel Horan[2]
[1]Agricultural and Bioresources Engineering Federal University of Technology, Minna Niger State Nigeria.
[2]Public Health Engineering, School of Civil Engineering, University of Leeds, Leeds United Kingdom
[1]cynthia.okoro@futminna.edu.ng , [2]n.j.horan@leeds.ac.uk

Abstract— **Microalgae are a good alternative to fossil fuel for energy generation. *Chlorella vulgaris* (*C.vulgaris*) strain of the microalgae was cultivated on liquor from chemically treated sewage sludge samples, to estimate the economic worth of sludge liquor; from the anaerobic digestion of the generated biomass. Sample1 was conditioned with zetag66 polyelectrolyte (control sample), samples 2 and 3 were treated with lime at different concentrations and sample 4 was treated with ferric chloride. Biomass yield (dry solids) was 1.36 kg/m^3, 2.94 kg/m^3, 1 kg/m^3 and 0.87 kg/m^3 for media 1, 2, 3 and 4 respectively. The control had the highest energy and economic worth per m^3 of 3.07 kWh and £1.12 respectively. Media from lime treatment had the same energy and economic worth per m^3 of 2.51 kWh and £0.91 respectively. Media from ferric chloride treatment had the least energy and economic worth per m^3 of 1.81 kWh and £0.66 respectively. Sludge liquor has potential for wealth creation and sustainable energy generation even after chemical sludge treatment, when used to cultivate microalgae.**

Index Terms— ***C.vulgaris*, liquor, energy, anaerobic, digestion.**

I. INTRODUCTION

A. Energy sources and demand

Energy generation with minimal greenhouse gas emissions is of global importance. The comfortable exploitation of fossil fuel resources for primary energy needs has become burdensome. These resources are diminishing very fast [1] and their use negatively affects the environment at large; with increasing accumulation of carbon dioxide in the atmosphere [2]. Renewable energy from biomass are now being exploited for environmental and economic sustainability [3].

Biomass energy supply increased from 38 to 52EJ between 1990 and 2010 as a result of increased energy demand and policies developed to increase the use of renewable energy [4]. Biomass is at present the largest contributor of world renewable energy, with even more potentials to exploit for electricity, heat and vehicle fuels [5].

1) Microalgae usage

Microalgae are single-cell, photosynthetic organisms [6], with rapid growth rate and high energy content [6], [7]. Microalgae have been the focus of research towards biomass production for renewable fuels such as biodiesel, methane, biohydrogen and bioethanol [8]. Different media compositions are proposed and used for growing microalgae, relative to investigations of the chemical environment of the natural occurring microalgae [9].

Microalgae has been used for many purposes such as anaerobic digestion to biogas [10], gasification to methane or hydrogen, transesterification to biodiesel [1], [6], pyrolysis gas or liquid fuels and burning to generate heat and electricity [6].

B. Sewage sludge liquor and treatment

Currently in the UK about 1.2 megatonnes of dry solids of wastewater sludge is produced annually and approximately 6.5 megatonnes of dry soilds by the EU as a whole [11]. Sludge contains nutrients such as ammonia, potassium and phosphorus and can therefore be used on agricultural lands for soil enrichment. A number of approaches are used by different wastewater treatment plants (WWTPs) to reduce sludge volume; thereby minimising handling and cost of transportation, destroying pathogens in the sludge and also smell reduction [12].

Sludge treatment processes include thickening, dewatering, biological treatment (anaerobic digestion and composting), and chemical stabilization, thermal treatment and drying.

During sludge treatment liquor is produced. By convention sludge liquor is returned to the WWTPs [13], [14] and removal of ammonia and phosphorus is achieved during side stream wastewater treatment [15]. This causes an additional nutrient

load [16] of up to 25 percent [12], [14], [17] to 30 percent [13], [18]. The high cost involved in treating sludge liquor when returned to treatment works, led to the decision by most WWTPs to adopt sustainable and less expensive methods for treating sludge liquor [13].

The use of microalgae for wastewater treatment purposes began only in recent years (1970s) [19]. The eutrophication of many waters due to effluent discharges, opened up the potential for microalgae to be used in treating wastewater [19]. Cultivation of microalgae for biomass production can therefore be achieved from nutrients in the wastewater [15]. More so, the microalgae can be used to destroy pathogens in the wastewater, by increasing the pH when CO_2 is consumed
[1].

C. Anaerobic digestion of microalgae

Anaerobic digestion (AD) of microalgae can be economical for methane generation, using low cost microalgae cultivated on wastewater or harvested from water bodies [10]. Unlike biofuel production from algae that depends on the algae lipid content, methane generation process recovers more energy than available in cell lipid [20]. With a cell lipid content below 40%, AD proves to be the optimal choice in terms of energy balance [20]. Generation of biogas by AD eliminates a lot of energy intensive steps such as drying and extraction [10].

Biochemical composition of biomass affects methane and biogas yield from AD and the amount of VS destroyed during AD is critical to the entire process. These factors put some huddles in biogas production from algae biomass because algae have limited biodegradability [10], [20]. For most macro- and micro-algae, only about 20 to 60% VS destruction can be achieved and as a result of algae cell wall, there is limited accessibility of enzymes to the substrates [10]. Therefore biodegradation is limited; leading to a longer retention time of about 20 to 30 days for the conventional AD process [10]. Also high cellular protein leads to release of ammonia, which in turn could result in toxicity [20].

The AD of microalgae for biogas can also be combined with the production of biodiesel using the post-extracted algal biomass [10], [20]. It was reported that the conversion of low lipid content algae to biogas has more potential energy than its conversion to biodiesel alone, while a combination of both yields the greatest energy output [21].

II. MATERIALS AND METHOD

A. Sludge sampling and liquor media preparation

Sewage sludge (combination of primary and secondary sludge) was obtained from Esholt wastewater treatment plant, UK. The sludge samples were chemically treated before centrifuging to obtain the supernatant. The first sample was prepared by adding 0.6 g zetag66 polyelectrolyte per litre of sludge; this served as the control sample. Polyelectrolyte was used to enhance dewatering. The second and third samples were prepared by adding lime at different concentrations in order to optimize the phosphorus distribution to the sludge cake. Optimization was done at varying concentrations, but only the liquor from the highest and least concentrations of lime was used for microalgae cultivation. Therefore the second sample was prepared using 27.5 g lime per litre of sludge, while the third was prepared using 1.28 g of lime per litre of sludge. And lastly the fourth sample was prepared by adding 35 g of ferric chloride to the sludge to also enhance the phosphorus distribution to the sludge cake. The samples were labelled sample1, sample2, sample3 and sample4 respectively.

Sludge samples were centrifuged using Eppendorf centrifuge5810 at 4000 revolutions per minute (rpm) for 30 minutes. Liquor samples obtained were too concentrated; still having high amount of suspended solids. As this was assumed to inhibit adequate light penetration for *C.vulgaris* photosynthesis, each sample was further centrifuged using 50 ml centrifuge tubes at 4000 rpm for 40minutes. The supernatant was then filtered using a GF/C 90mm filter paper; to obtain clear liquor. Liquor samples were thereafter autoclaved at 120˚C and 2.5 bar pressure, in order to destroy any bacteria or pathogen that could inhibit the growth of the algae in the media. Samples were then cooled to room temperature.

Liquor samples were characterized for phosphate, pH, alkalinity and ammonia-nitrogen; these parameters influenced the dilutions done for each sample and the volume of *C.vulgaris* used in preparing the culture media.

B. C.*vulgaris cultivation in the liquor media*

The liquor samples prepared from the sludge were used to cultivate microalgae. *C.vulgaris* was obtained from a culture in Bolds Basal Media (BBM) in the University of Leeds laboratory, on the 4th day of culture (at the exponential growth phase). Two hundred (200) ml of liquor media was prepared from each sample and cultivated in 500 ml clogged conical flasks. Aeration was achieved by the shaking action of the incubator. Equal volume of *C.vulgaris* (50 ml) was used in each media. Media1 was prepared using 1/15 ml dilution of sample1, media2 was prepared using 1/3 ml dilution of sample2, media3 was prepared using 1/16.7 ml dilution of sample3 and lastly media4 was prepared using 1/30 ml dilution of sample4. The dilutions were done in order to control the nutrient in each culture. The overall percentage ratio of liquor to *C.vulgaris* in each media was 5:25, 25:25, 4.5:25 and 2.5:25 for media1, media2, media3 and media4 respectively. The cultures were then cultivated in the INFORS HT incubator for 14 days. Total suspended solids (TSS) and volatile suspended solids (VSS) tests were carried out on the media during the cultivation period. These tests measure the biomass growth in the media.

1) Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) Analysis a) Materials

The ESS method 340.2 [22] was used to determine TSS and VSS. Materials used for these tests include filtration apparatus, 9 cm glass fibre filter paper, vacuum pump, oven, furnace, desiccators and watch glass.

b) Procedure

The glass fibre filter paper was weighed and recorded. The filter paper was then placed on the filtration apparatus, with the vacuum pump in place. Ten (10) ml of sample was filtered through. The filter paper was then placed in an oven drying tray and dried in the oven (Gollenhamp Hotbox oven) at 105°C for 24 hours. The filter paper was then placed in a crucible and kept in a desiccator to cool. The filter paper was weighed at an interval of 10 minutes, until a constant weight was recorded. The TSS (mg/l) was then calculated using the formula below;

$$TSS = \frac{dried\ wt.(mg) - initial\ wt.(mg)}{volume\ used\ (ml)} \times 1000. \quad (1)$$

The volatile suspended solid (VSS) was then determined by placing the filter paper from the TSS in a crucible and transferring it to a furnace at 560˚C for 2 hours, to remove all volatiles. Afterwards it was allowed to cool in a desiccator and weighed continuously at an interval of 10 minutes, until a constant weight was observed. The VSS (mg/l) was then calculated by the equation;

$$VSS = \frac{dried\ wt.from\ TSS\ (mg) - final\ wt.(mg)}{volume\ used\ (ml)} \times 1000. \quad (2)$$

C. C.vulgaris growth rate and biomass yield in liquor media

Using a spectrophotometer, the absorbance; optical density at 540nm wavelength (OD_{540}) was carried out on each media to determine growth rate. Absorbance has been used to estimate growth rate in microalgae cultivation [1], [19], [23]. Five point measurements were done on day1, day4, day7, day11 and day14 and the increase in the absorbance was used to determine the growth rate of the algae in each media. The growth rate was then calculated using the equation below,

$$Growth\ rate\ (/day) = (InOD_t - InOD_0)/t. \quad (1)$$

for

Where OD_t is the optical density at day t, OD_0 is the initial optical density and t is the number of days.

$$VSS\ Yield\ (\%) = \frac{VSS_f - VSS_i}{TSS_f - TSS_i} \times 100. \quad (5)$$

Where TSS_f and VSS_f are the TSS and VSS values after 14 days respectively and TSS_i and VSS_i are the initial TSS and VSS values after media preparation before culture.

C.vulgaris yield per m^3 of media was also calculated for all liquor media using the equation below;

$$Y_d = \frac{TSS_f - TSS_i}{1000} \quad (6)$$

At the end of the 14 days cultivation period, the percentage biomass yield was calculated as a function of the total suspended solids (TSS);

$$TSS\ Yield\ (\%) = \frac{TSS_f - TSS_i}{TSS_f} \times 100. \quad (4)$$

The Volatile suspended solids (VSS) yield was calculated as a percentage of the TSS using the equation;

Methane Potential (BMP) test was carried out on the AD of *C.vulgaris*. The result was then used to estimate how much methane can be obtained from *C.vulgaris* cultivated in the liquor media.

$$Y_M = BMP \times Biomass\ VSS\ (kg/m^3 media) \quad (8)$$

Where Y_M = Methane yield (m^3/m^3media), BMP = biochemical methane potential (m^3methane/kgVSS_{added})

The energy worth of 1 m^3 of liquor media was then analysed. One (1) Nm^3 (normalized cubic metre) of methane is reported to be equivalent to 9.97 kWh of energy [24]. Therefore from the methane yield calculated, the amount of energy per m^3 of media was estimated by the following equation;

$$Energy\ (kWh/m^3 media) = 9.97 \times Y_M. \quad (9)$$

The use of AD technology to generate energy in the UK has been greatly encouraged by some government incentives. These incentives are avenues to generate income for the energy derived from any AD plant. Figure 1 shows the options for the methane generated from AD and their respective incentives.

For the sake of this study, the use of methane for income generation from combined heat and power was analysed. The Department for food and rural affairs, UK (Defra) [25] reported that using AD to generate renewable energy attracts 2 250 kW and 500kW [25]. Therefore 14p/kWh was used RoCs/MWh. A typical value of £80/MWh was assumed for 1 RoC. FiTs for small scale AD with capacity of up to 250 kW is 14p/kWh and 13p/kWh is provided for capacities between

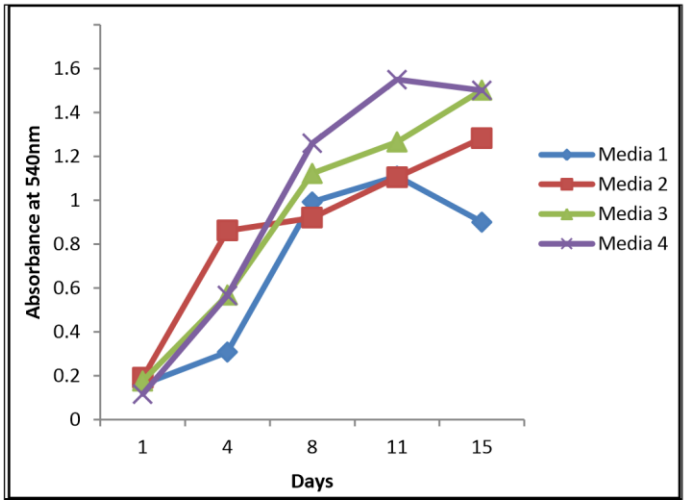
Where Y_d = Biomass yield (kgDS/m^3media), TSS_f = final TSS (mg/l) and TSS_i = initial TSS (mg/l).

$$Y_v = \frac{VSS_f - VSS_i}{1000} \quad (7)$$

Where Y_v = biomass yield (kgVS/m^3media), VSS_f = final VSS (mg/l) and VSS_i = initial VSS (mg/l)

D. Methane and energy worth of C.vulgaris from liquor media

The theoretical methane that can be achieved from *C.vulgaris* biomass obtained was calculated. A Biochemical FiTs. Lastly the RHi provides 6.5p/kWh over a 20 year period for biogas combustion in installations below 200kWh and biomethane injection into the gas grid at all scales [25]. These incentives were then used to estimate the financial worth of the methane generated from liquor media.



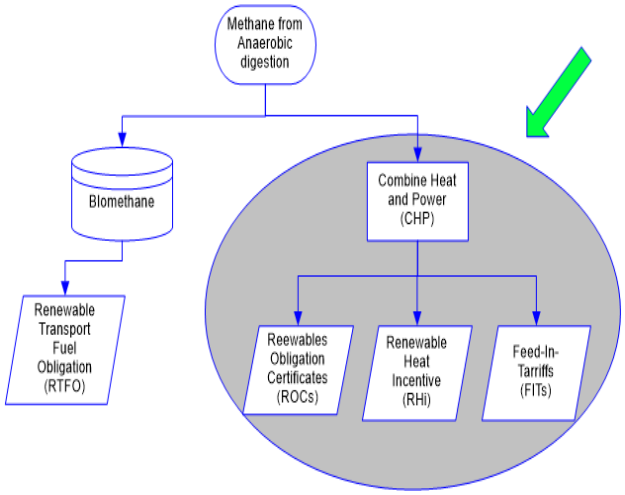


Fig. 1. Options for Methane from AD and their respective incentives in the UK.

III. RESULTS AND DISCUSSION

A. C.vugaris cultivation and growth rate in liquor media

For each media, a different amount of ammonia and phosphate was taken up by *C.vulgaris*. The initial and final media characteristics are presented in table 1.

The growth rate of *C.vulgaris* calculated during the exponential growth phase and the biomass yield are presented in table 2. In contrast to the low specific growth rates of *C.vulgaris* observed in all media presented in table 2, an average specific growth rate of 0.948 d^{-1} for *Chlorella sp.* when cultivated in sludge liquor was reported [19].

Low growth rates have however been recorded for *Chlorella sp.* cultivated in different media types; a specific growth rate for *Chlorella sp.* of 0.035 day^{-1}, when cultivated on filtrate from

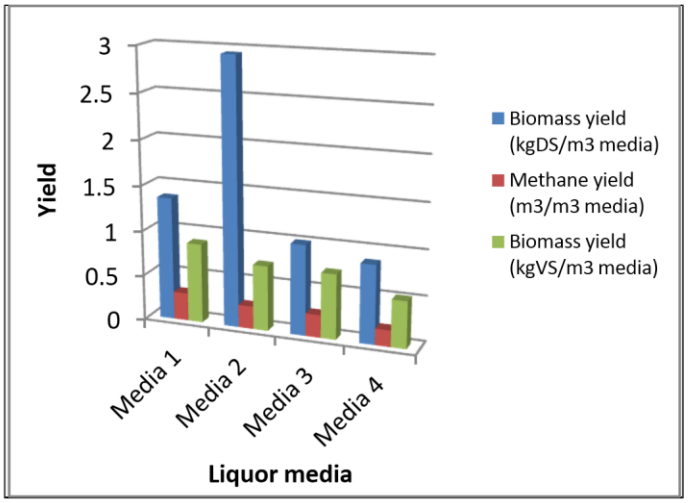


Figure 3. Methane yield per cubic meter of liquor media in comparison with biomass yield.

anaerobic digestate [26], growth rate for *Chlorella sp.* of 0.45 day^{-1} when cultivated in a modified Zarrouk medium was also recoded [27] and a low growth rate for *C.vulgaris* of 0.103 d^{-1} at the exponential growth phase, when cultivated in BG11 medium [15]. The low growth rate recorded in this study can be said to be influenced by the N/P ratios of the media in the range of 13.3 – 42.9 compared to the suggested optimal range of 6.8 – 10. Clearly only media1 had close to the suggested range.

Media1 and media4 in fig. 2 demonstrate a standard growth curve of *C.vulgaris* in batch cultivation. The trend of media2 and 3 shows that if the cultivation was continued after the 14 days cultivation period, more growth would be realised from the media. The high absorbance observed in the media from chemical treatments (2, 3 and 4) could be due to the residual effect of the chemicals used in the three media during sludge treatment.

The outcome of *C.vulgaris* cultivation showed that media2 with equal amount of *C.vulgaris* and liquor had the highest biomass yield (dry solids) of 2.94 kg/m^3 followed by media1 yielding 1.36 kg/m^3, media 3 yielded 1 kg/m^3 and finally media4 yielded 0.89 kg/m^3. This follows the increasing order of *C.vulgaris* in the media; that is media2 had the least amount of *C.vulgaris*, followed by media1, media3 and

1.8

Fig. 2. *C.vulgaris growth curve in liquor media*

Table 1. Initial and final characteristics of media samples

	Media 1		**Media 2**		**Media 3**		**Media 4**	
	initial	final	initial	final	initial	final	initial	final
pH	9.39	7.92	9.09	7.82	9.76	7.82	7.50	7.82
Alkalinity ($mgCaCO_3/l$)	1730.0	215.0	2805.0	2245.0	1985.0	2507.7	789.2	1045.0
Ammonia (mg/l)	212.8	5.6	212.8	33.6	302.4	33.6	515.2	44.8
Phosphate (mg/l)	16.00	1.36	7.50	0.80	10.25	1.20	12.00	2.00
TSS (mg/l)	60	1420	100	3040	200	1200	410	1280
VSS (mg/l)	450	1330	710	1430	400	1220	470	990

media4 in that order. Therefore increasing the amount of *C.vulgaris* in sludge liquor media will gave rise to decrease in the biomass yield (dry solids). As all media had suitable pH and no media was starved of nutrient; having sufficient ammonia and phosphate, the ratio of *C.vulgaris* to liquor can be said to be the primary influence for the biomass yield observed.

B. Methane yield and energy potential following anaerobic digestion of C.vulgaris

The amount of volatile solids generated is of great importance. Media1 had the highest amount of volatile solids of 0.88 kg/m^3, media2 and 3 had the same amount of volatile solids of 0.72 kg/m^3 while media3 had the least amount of volatile solids of 0.52 kg/m^3. The amount of methane that could be generated from *C.vulgaris* biomass cultivated in the control media was calculated as described in Eq. 6 and Eq 7.
The methane potential from the BMP test on *C.vulgaris* was 0.35 $m^3/kgVS_{added}$. A similar BMP of 0.31 $NLmethane/gVS_{added}$ for *Chlorella* cultivated on sludge liquor was recorded [26]. On the other hand, a low methane yield from 100% *Chlorella* digestion of 126 $mL\text{-}CH_4/gVS_{fed}$ have also been recorded [27].

The essence of estimating the methane yield is to know the energy and economic worth of sludge liquor when *C.vulgaris* is cultivated on it. Since 1 Nm^3-methane is equivalent to 9.97 kWh of energy [24], [28], a sum of 36.5p/kWh is obtainable from energy generated, using the incentives from fig. 1.

Figure 3 shows that media1 had the highest methane yield of 0.308 m^3/m^3media, media2 and media3 from the lime treatment liquor both yielded 0.252 m^3/m^3media, while media4 had the least methane yield of 0.182 m^3/m^3media.

With energy equivalent of 9.97 kWh per Nm^3-methane, dewatering liquor from WWTPs yields up to 3.07 kWh/m^3media. Therefore in cultivating *C.vulgaris* for methane generation from AD, more attention should be paid to factors that enhance volatile solids than dry solids yield.

Raw liquor from dewatering process has good methane and energy potential. Liquor from lime treatment would generate about the same amount of methane (regardless of the amount used); this should be considered when using such treatment. Ferric chloride treatment would have significant effect on the methane generated from the liquor media.

Table 2. C.*vulgaris* specific growth rate and percentage biomass yield after 14 days of cultivation

Sample	**Growth rate (/d)**	**Biomass yield (%)**	
		TSS	VSS (% of TSS)
Media 1	0.228	95.8	65
Media 2	0.020	96.7	24
Media 3	0.185	83.3	72
Media 4	0.231	68.0	60

The resultant energy and economic worth of 1 m^3 of each liquor media used have been presented in table 3.

The conventional oxidation of ammonium ions to nitrate (NO_3) and then denitrification to nitrogen gas as a treatment option in WWTPs require a lot of energy for pumping and aeration and therefore high cost. For this process, 4.57 gO_2/gNH_3-N is required for complete oxidation. It therefore means that for the 2240 $mgNH_3$-N/l (2.24 gNH_3-N/l) in the liquor obtained from sample1, an additional 10.24 gO_2/l (10.24 kgO_2/m^3) is required. On the other hand, the photosynthetic activity of the microalgae produces oxygen. A maximum of 10 $gO_2\,m^{-3}\,min^{-1}$ (14.4 $kgO_2\,m^{-3}\,d^{-1}$) can be generated by microalgae in a typical photobioreactor [29]. Therefore, 141% of the oxygen required to treat the liquor by conventional process would be produced daily. This can be extracted and used in WWTPs for other necessary biological processes. The energy and cost of aeration as required by the conventional process would therefore be greatly saved.

Also 1 m^3 of media has a financial worth of £1.12. The ammonia available in the liquor from sample1 is about 11 times the ammonia required to prepare media1. Therefore, 11 times the financial worth of 1 m^3 of media can be generated. In essence, the raw liquor from sludge dewatering is worth about £12.32.

Chemical precipitation of nutrients into the sludge cake depends on specific soil characteristics and the availability of

Table 3. Energy and economic value of $1m^3$ liquor media and the respective ammonia and phosphate required.

Sample	Energy yield (kWh/m^3media)	Economic worth (£/m^3media)	Ammonia required (mg/l)	Phosphate required (mg/l)
Media 1	3.07	1.12	207.2	14.64
Media 2	2.51	0.91	179.2	6.7
Media 3	2.51	0.91	268.8	9.05
Media 4	1.81	0.66	459.2	10

plant to use up the nutrient [17]. Therefore if there is insufficient landbank or where there are stringent regulations regarding the spreading of sludge cake on land, the process becomes futile. As such since *C.vulgaris* showed an impressive growth with the highest economic value in the raw liquor media, it would be better to use just microalgae for treating sludge liquor. However, wherever it is necessary to use chemicals, only little chemical dosing should be employed in order to save cost.

IV. CONCLUSION

The energy and revenue potential of the biomass generated from cultivating *C.vulgaris* on sludge liquor makes the use of *C.vulgaris* for sludge liquor treatment more economical than conventional nutrient removal processes.

REFERENCES

[1] F. Ahmad, A. Khan, and A. Yasar, "The potential of Chlorella vulgaris for wastewater treatment and biodiesel production," *Pakistan J. Bot.*, vol. 45, no. 1, pp. 461–465, 2013.

[2] Y. P. Li, G. H. Huang, and M. W. Li, "An integrated optimization modeling approach for planning emission trading and clean-energy development under uncertainty," *Renew. Energy*, vol. 62, pp. 31–46, Feb. 2014.

[3] B. Esteban, J.-R. Riba, G. Baquero, R. Puig, and A. Rius, "Environmental assessment of small-scale production of wood chips as a fuel for residential heating boilers," *Renew. Energy*, vol. 62, pp. 106–115, Feb. 2014.

[4] World Energy Council, "World Energy Resources 2013," London, 2013.

[5] World Energy Council, "World Energy Resources: Bioenergy," London, 2013.

[6] A. L. Stephenson, E. Kazamia, J. S. Dennis, C. J. Howe, S. a. Scott, and A. G. Smith, "Life-Cycle Assessment of Potential Algal Biodiesel Production in the United Kingdom: A Comparison of Raceways and Air-Lift Tubular Bioreactors," *Energy & Fuels*, vol. 24, no. 7, pp. 4062–4077, Jul. 2010.

[7] A. Singh, P. S. Nigam, and J. D. Murphy, "Renewable fuels from algae: an answer to debatable land based fuels.," *Bioresour. Technol.*, vol. 102, no. 1, pp. 10–6, Jan. 2011.

[8] K.-L. Yeh and J.-S. Chang, "Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga Chlorella vulgaris ESP-31.," *Bioresour. Technol.*, vol. 105, pp. 120–7, Feb. 2012.

[9] R. K. Mandalam and B. O. Palsson, "Elemental balancing of biomass and medium composition enhances growth capacity in high-density Chlorella vulgaris cultures.," *Biotechnol. Bioeng.*, vol. 59, no. 5, pp. 605–611, Sep. 1998.

[10] P. Bohutskyi, M. J. Betenbaugh, and E. J. Bouwer, "The effects of alternative pretreatment strategies on anaerobic digestion and methane production from different algal strains.," *Bioresour. Technol.*, vol. 155, pp. 366–72, Mar. 2014.

[11] N. K. Garg, "Multicriteria Assessment of Alternative Sludge Disposal Methods Neeraj Kumar Garg," University of Strathclyde, 2009.

[12] T. Evans, "SEWAGE SLUDGE : Operational and Environmental Issues," United Kingdom, 2011.

[13] F. N. Ocansey, "New trends in the treatment of reject water from dewatering of sludge," University of Lund, 2005.

[14] T. Evans, "Sludge treatment and dewatering," *water sewarage J.*, vol. 4, pp. 39–41, 2011.

[15] R. Boonchai, G. T. Seo, D. R. Park, and C. Y. Seong, "Microalgae Photobioreactor for Nitrogen and Phosphorus Removal from Wastewater of Sewage Treatment Plant," *Int. J. Biosci. Biochem. Bioinforma.*, vol. 2, no. 6, pp. 407–410, 2012.

[16] H. W. Demooij and G. Thomas, "Ammoniacal Nitrogen Removal from Sludge Liquors – Operational experience with the DEMON process Keywords The Apeldoorn Project," in *15th European Biosolids and Organic Resources Conference*, 2010.

[17] N. Jardin, D. Thöle, and B. Wett, "Treatment of sludge return liquors: experiences from the operation of full-scale plants," *Proc. Water Environ. Found.*, pp. 5237–5255, 2006.

[18] D. Willie and G. Reitsma, "One-Step ANAMMOX Process: a sustainable way to remove ammoniacal nitrogen," United Kingdom, 2011.

[19] L. Wang, M. Min, Y. Li, P. Chen, Y. Chen, Y. Liu, Y. Wang, and R. Ruan, "Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant.," *Appl. Biochem. Biotechnol.*, vol. 162, no. 4, pp. 1174– 86, Oct. 2010.

[20] B. Sialve, N. Bernet, and O. Bernard, "Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable.," *Biotechnol. Adv.*, vol. 27, no. 4, pp. 409–16, 2009.

[21] R. Harun, M. Davidson, M. Doyle, R. Gopiraj, M. Danquah, and G. Forde, "Technoeconomic analysis of an integrated microalgae photobioreactor, biodiesel and biogas production facility," *Biomass and Bioenergy*, vol. 35, no. 1, pp. 741–747, Jan. 2011.

[22] USEPA, "ESS method 340.2," United States, 1993.

[23] F. Ji, Y. Liu, R. Hao, G. Li, Y. Zhou, and R. Dong, "Bioresource Technology Biomass production and nutrients removal by a new microalgae strain Desmodesmus sp . in anaerobic digestion wastewater," *Bioresour. Technol.*, vol. 161, pp. 200–207, 2014.

[24] N. Horan, "Basic Terminology. CIVE5532M Wastewater and organic waste management," *Univ. Leeds.*, 2013. [25] Defra, "Anaerobic Digestion Strategy and Action Plan," 2011.

[26] T. Hidaka, K. Inoue, Y. Suzuki, and J. Tsumori, "Bioresource Technology Growth and anaerobic digestion characteristics of microalgae cultivated using various types of sewage," *Bioresour. Technol.*, vol. 170, pp. 83–89, 2014.

[27] M. Wang, A. K. Sahu, B. Rusten, and C. Park, "Bioresource Technology Anaerobic co-digestion of microalgae Chlorella sp . and waste activated sludge," *Bioresour. Technol.*, vol. 142, pp. 585–590, 2013.

[28] N. Horan, "The products: Biogas and Digestate. CIVE5532M Wastewater and organic waste management," *Univ. Leeds.*, 2013.

[29] Y. Chisti, "Biodiesel from microalgae.," *Biotechnol. Adv.*, vol. 25, no. 3, pp. 294–306, 2007.