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Microbiological Quality and Physico-Chemical Composition of Domestic and Restaurant Wastewater as a Media for Microalgae Cultivation

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Abstract

Mass cultivation of algae has suffered series of set-backs and one of its challenges in media development. In this study, microbiological and physicochemical quality of the different domestic and restaurant wastewater were ascertained as potential feedstock for microalgae cultivation. The samples were prepared in 180:20, 160:40, 140:60, 120:80, and 100:100 ratios using pond water and mixture of domestic and restaurant waste water respectively. The blooming process was carried out under natural illumination for seven (7) days and the optical density (abs) and cell dry weight (mg/l) were monitored. The physico-chemical results showed that the wastewater were composed of vital inorganic nutrients such as nitrate (19.43±0.10, 17.48±0.56mg/l); sulphate (32.43±0.29, 33.38±0.21 mg/l); phosphate (3.27±0.12, 2.76±0.03 mg/l); ammonia (2.29±0.01, 2.15±0.03 mg/l); TDS (236.67±2.99, 236.33±2.91 mg/l); BOD (38.11±0.30, 39.66±0.70 mg/l); COD (283.95±0.25, 323.70±3.28 mg/l) for cultivation of microalgae. The total heterotrophic count for restaurant and domestic wastewater samples ranged from $1.06x10^6$cfu/ml to $1.19x10^6$cfu/ml and $1.0x10^4$cfu/ml to $1.2x10^4$cfu/ml for bacteria and fungi respectively. The bacterial isolates identified were *Bacillus* sp., *Echerichia coli*, *Staphylococcus* sp., *Pseudomonas* sp. and *Salmonella* sp. while fungal isolates were *Penicillium* sp., *Aspergillus* sp. and *Mucor* sp. The best optimal wavelength (620nm) was selected for growth monitoring at a ratio of 120:80 for restaurant: domestic wastewater. The inexpensive nutrient media composition of the wastewater could be positively harnessed in an integrated waste-microalgae mass cultivation process.

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Mass cultivation,
Microalgae,
Microbiological,
Physicochemical,
Wastewater.

Introduction

The demand for food and energy supply has doubled within the last six years (Batista *et al.*, 2015). This is because of the current growing trend in the population explosion, industrial revolution, and eco-deterioration. One of the challenges facing the world is shortage of food and energy supplies and the decline in the quality of environmental conditions and the link between them and their solution is not far-fetched. These current challenges suggest a vibrant fusion between industrial waste management, phycology and algal biotechnology, as one way of solving these problems. Diversification in the use of biological resources includes saline and fresh water sources, production of biofuels to meet growing demand and decline of non-renewable and conventional fuels (Agwa *et al.*, 2012).The application of microalgae in waste water treatment is a profitable tool in the drive to meet an eco-friendly, cost-effective, safer and cheaper material-use. Advantages of algal biotechnology include

minimal-agitation technologies, reduction in green-house gas emission, chemical free processes, minimal sludge formation, these are key to a sustainable ecosystem (Batista *et al.,* 2015). This is because the biomass product can be diversified into nutraceutical, pharmaceutical and bioactive substances (Razzak *et al.,* 2013). Furthermore, mechanized and re-circulatory processes have been identified to offer both ecological and several economic benefits (Brennan and Owande, 2010). The re-use ability of the waste water from the production of higher energy products, offers a cheaper and less intensive demand.

Currently, biofuel technologies employed all over the world have put more interest in plants. Microalgal alternatives due to their growth rates and yield have been identified to surpass these sources, to ease off problems and their culturing as well as lack of pressure on arable lands. Microbial biotechnology is expected to be generally acceptable, when the process is clean, sustainable and of reliable techniques, thus meeting the daily needs of human populace. Use of microalgae in an inexpensive locally formulated media has been identified as a sustainable alternative for biotechnology production (Agwa *et al.,* 2012). Microorganisms and biosystems can be utilized to generate electrical energy to power appliances (Logan *et al.,* 2008). Microalgal biomass possesses unique, photosynthetic machinery, can be used as a tool for fermentation and commercial production of biohydrogen, because of its rich carbohydrate content (Chader *et al.,* 2011).

Owing to its rapid growth rate and productivity, algae have been described as an inexhaustible source of renewable biomass-resource and lipid (Feng *et al.,* 2011). Microalgae have been identified as an effective tool to sequester solar radiation and yield huge biomass with little or no harm on the ecosystem (Agwa *et al.,* 2013). The ability of this group of microalgae to adapt to extreme conditions while accumulating high value compounds has been applauded (Chader *et al.,* 2011).

And Metcalf and Eddy, (1991) reported that treatment of wastewater implies removal of unwanted components to rescue the environment from decay and promote algal multiplication. Chisti, (2007) noted that algae can accumulate upto 80% by weight of their cell mass and form biomass for diverse biotechnological processes. Their nutritional mechanism has the ability to utilize various nutrients as heterotrophs, mixotrophs and photoheterotrophs sources. They have the ability to use nitrates and phosphates within medium to accumulate huge biomasses (Chader *et al.,* 2011), serving as feedstock for biofuel production. Biotechnological purposes have employed different wastewater treatment and re-use for different biological processes by removing the harmful contents and preserve the ecosystem (Chader *et al.,* 2011). Li *et al.,* (2011) reported that the productivity of most microalgae is up to 20-40 times more than most terrestrial crops. In an earlier study, Agwa *et al.,* (2013) reported that *Chlorella vulgaris* is the most popularly studied alga, with several biotechnological potentials steming from miconucles and tapping into its immediate huge financial turn-over. The presence of these organics accounts for nutritional components that could be harnessed in the growth and synthesis of high-value algal compounds.

Materials and Methods

Sample collection and physical examination of the sewage

A chemically clean container was used to collect waste-water from three domestic and restaurant discharge points within Choba Port Harcourt.

Physico-chemical analysis of sewage

The waste-waters (domestic and restaurant) were subjected to A.O.A.C (2000) and APHA, (2000) standard determination of their particulate composition.

Microbiological analyses

Serial dilution of domestic and restaurant wastewater samples were carried out by standard microbiological techniques. The inoculums were aseptically and evenly spread on the surface of the Nutrient Agar for bacteria and Potato Dextrose Agar for fungi in duplicate.

The plates were incubated aerobically at 37^0C for 24h and 28 ± 2^0C for 96h for bacteria and fungi respectively (Uzoigwe and Agwa, 2012).

Optimal Wavelength Selection

The optimal wavelength of the medium was obtained by scanning the waste water (effluents) from low to high wavelength of the spectrophotometer (model/y). The best optimal wavelength was determined from the point of least absorbance (Mogany, 2014 and Wang *et al.,* 2008).

Cultivation of Microalgae in the Ratio of Domestic and Restaurant Wastewater

Modified method of Agwa *et al.*, (2013) was adopted for the study. The ratio of 180:20, 160:40, 140:60, 120:80, and 100:100 using pond water and mixture of domestic and restaurant waste water were assessed to determine the best ratio for monitoring the growth of the microalgae. Novel synthetic media was used as positive controls and the un-inoculated waste water was used as negative controls. The optimum wavelength was used to monitor the growth of the microalgae in the media, in which about 10ml of the broth was poured into a cuvette and the absorbance read-off from the spectrophotometer.

The Cell Dry weight approach was used to determine the algal biomass yield. Ten milliliters (10ml) of the culture broth was centrifuged at 4,500xg for 15 minutes, washed with physiological saline three times, the pellets were dislodged and then poured on a pre-weighed Whatman filter and the sample was dried at 50^0C in a hot oven to constant weight then brought to room temperature in a desiccator. The net dry cell weight was determined by measuring the arithmetic difference of final weight of the filter paper and the initial weight.

Statistical Analysis

The statistical package for social sciences (SPSS 20.0 version) provided the platform for Duncan multiple range test, sheffe and tukey-HSD tools used to compare the parameters and locate points of significance at a confidence level of P= 0.05 (95%).

Results and Discussion

The physicochemical composition of wastewater, revealed that the pH of the restaurant wastewater had significant difference between the samples, 6.55±0.04, 6.12±0.10 and 6.30± 0.21, this was observed to be higher than FEPA standards (Table 1).

While that of domestic wastewater varied from 6.15± 0.08 and 6.39 ± 0.05 for DA and DC were also observed to be higher than the FEPA standard except for sample DB which was lower 5.73 ±0.09 (Table 2). The dissolved oxygen for domestic wastewater ranged from 52.895.73 ±0.11 ppm, 33.625.73 ±0.24 ppm and 54.245.73 ±0.25 ppm, while that of the restaurant waste water was observed to be 38.45±0.41, 62.94± 1.09 and 39.36± 0.83 ppm. The total nitrogen was 1.52±0.06, 2.18±0.09 and 1.36± 0.03 ppm for RA, RB and RC respectively. The domestic wastewater were 2.06 ± 0.03, 1.41 ± 0.03 and 2.91 ± 0.01for DA, DB and DC respectively (Table 1 and 2). The total heterotrophic counts for bacterial and fungal counts for domestic wastewater were $1.19x10^6$cfu/ml and $1.2x10^4$cfu/ml while $1.06x10^6$cfu/ml and $1.0x10^4$cfu/ml for bacterial and fungal count from restaurant wastewater samples. The total heterotrophic count for bacterial and fungal isolates (Fig.1). The wavelength selection was observed in domestic and restaurant wastewater. The response of varying wavelength and optical densities. The results demonstrate

Wastewater generation in Nigeria is one challenge that has remained uncontrolled as they are being discharged into the environment without treatment. Quite a number of domestic and restaurant activities generate wastewater with high pathogenic bacterial communities. This has necessitated their exploration as a veritable source of nutrients for industrial application. Enitan *et al.*, (2015) reported that the physicochemical composition of wastewater can be harnessed as veritable sources of nutrient for commercialization. Furthermore, Agwa *et al.*, (2013) reported that huge amount of organic and inorganic nutrients are tied to agrowaste materials especially the fecal droppings, which serve as reservoir of nutrients for various biological processes. Results of the physic-chemical analysis revealed that the pH of the restaurant and domestic wastewater were recorded as 6.15± 0.08, 6.39± 0.05, 6.55±0.04 and 6.12±0.10, 6.30± 0.21, 5.73 ±0.09 respectively. The pH recorded in this study agrees with the findings of Benit and Roselin (2015) who reported pH of sewage water which range from 6.3-7.3.The pH was observed to be slightly acidic and allows for proliferation of wide arrays of microorganisms. The pH of a medium adversely affected the bioavailability profile of the nutrients and its accessibility to target microbial communities. That suggests that extreme pH conditions may hinder the usefulness of any wastewater (Sunny and Mathai, 2013).Patil *et al.*, (2008) reported that the pH of any wastewater can be correlated with the presence of carbonates, bicarbonates and other components that interacts with the electrical conductivity. Kirkham (2006) opined that pH affects the bioavailability of trace minerals which could support growth of fastidious microbes. The salinity and electrical conductivity of ions and radicals could enhance the use of the wastewater as a veritable source of nutrients.

Table.1 Physicochemical composition of restaurant wastewater

Parameter	Restaurant Wastewater (RA)	Restaurant Wastewater (RB)	Restaurant Wastewater (RC)	FEPA	WHO STANDARD
pH	6.55±0.04[b]	6.12±0.10[a]	6.30±0.21[a]	6.0	6.5-8.5
EC (µS/cm)	24.93±7.45[a]	271.00±6.35[b]	240.93±2.60[c]	120	120
Ca^{2+} (ppm)	49.62± 0.37[a]	32.57±0.59[b]	56.48±0.43[c]	60	60
Mg^{2+} (ppm)	39.27±0.48[a]	25.41±0.50[a]	4.681± 0.21[b]	0.05	0.05
DO (ppm)	38.45±0.41[a]	62.94± 1.09[b]	39.36± 0.83	4	4
BOD (ppm)	24.51± 0.21[a]	38.11± 0.30[b]	26.38± 0.20[a]	6	6
COD (ppm)	174.61± 0.35[a]	283.95 ± 0.25[b]	184.05± 1.58[c]	430	250
Nitrate (ppm)	19.43± 0.10[a]	13.60± 0.23[b]	16.52± 0.11[c]	10	10
Phosphate(ppm)	3.27± 0.12[a]	2.08±0.02[b]	3.04± 0.03[a]	7	1.0
Sulphate (ppm)	30.37± 0.45[a]	25.27± 0.08[b]	32.64±0.29[a]	500	500
TN (%)	1.52±0.06[a]	2.18±0.09[b]	1.36± 0.03_a	5	5
TDS (ppm)	162.72± 1.00[a]	236.67± 2.99[b]	158.46±0.92[c]	500	500
TSS (ppm)	126.10± 3.35[a]	156. 87±10.90[b]	118.28±0.08[a]	210	210
TOC (%)	7.17± 0.04[a]	2.17± 0.03[a]	6.07± 0.09[a]	0.05	0.05
Carbonates (ppm)	1.98± 0.00[a]	2.83 ± 0.19[aa]	1.64 ±0.039[a]	1.25	2
Salinity (ppm)	74.27± 0.55[a]	44.78 ± 0.54[b]	78.65±0.15[c]	90	100
Ammonia (ppm)	1.62 ± 0.02[a]	2.29 ± 0.01[a]	1.86± 0.03[a]	150	150

Means ±Standard Error; superscripts with the same alphabet in a given row are statistically insignificant at $p < 0.05$

Table.2 Physicochemical composition of domestic wastewater

Parameter	Domestic Wastewater (DA)	Domestic Wastewater (DB)	Domestic Waste water (DC)	DPR FEPA STANDARD	WHO STANDARD
pH	6.15± 0.08[a]	5.73 ±0.09[b]	6.39 ± 0.05[c]	6.0	6.5-8.5
EC(uS/cm)	339.08±8.87[a]	191.03 ± 2.59[b]	364.51 ± 6.50[c]	120	120
Ca^{2+}(ppm)	33-91±0.91[a]	56.77± 0.24[b]	39.04± 0.22[a]	60	60
Mg^{2+}(ppm)	27.53 ± 0.43[a]	39.40 ± 0.59[b]	26.07± 0.29[a]	0.05	0.05
DO(ppm)	52.89±0.11[a]	33.62 ± 0.24[b]	54.24 ±0.25	4	4
BOD(ppm)	36.70 ± 0.09[a]	23.77 ± 0.22[b]	39.66 ± 0.70[a]	6	6
COD(ppm)	314.89±2.92[a]	201. 14 ± 0.34[b]	323. 70 ± 3.28[a]	430	250
Nitrate(ppm)	14.73± 0.04[a]	17.48 ± 0.56[b]	13.67 ± 0.09[c]	10	10
Phosphate(ppm)	2.48 ± 0.01[a]	2.76 ± 0.03[a]	2.66 ± 0.03[a]	7	1.0
Sulphate (ppm)	26.7± 0.18[a]	33.38 ± 0.21[b]	28.54 ± 0.07[c]	500	500
TN (%)	2.06 ± 0.03[a]	1.41 ± 0.03[a]	2.91 ± 0.01[b]	5	5
TDS(ppm)	216.67±0.23[a]	150.23 ± 0.63[b]	236.33 ±2.91[c]	500	500
TSS(ppm)	154.31± 1.25[a]	113.34 ± 0.03[a]	146.33 ± 0.33[b]	210	210
TOC (%)	4.73 ± 0.04[a]	5.73 ± 0.09[b]	3.64 ± 0. 19[c]	0.05	0.05
Carbonates (ppm)	2.53± 0.11[a]	1.52 ± 0.09[b]	2.46 ± 0.03[a]	1.25	2
Salinity(ppm)	46.23 ± 0.61[a]	73.29 ±0.51[b]	52.41 ± 0.42[c]	90	100
NH_3(ppm	2.15±0.03[a]	1.82 ± 0.06[a]	2.07 ± 0.05[a]	150	150

Means ±Standard Error; superscripts with the same alphabet in a given row are statistically insignificant at $p < 0.05$

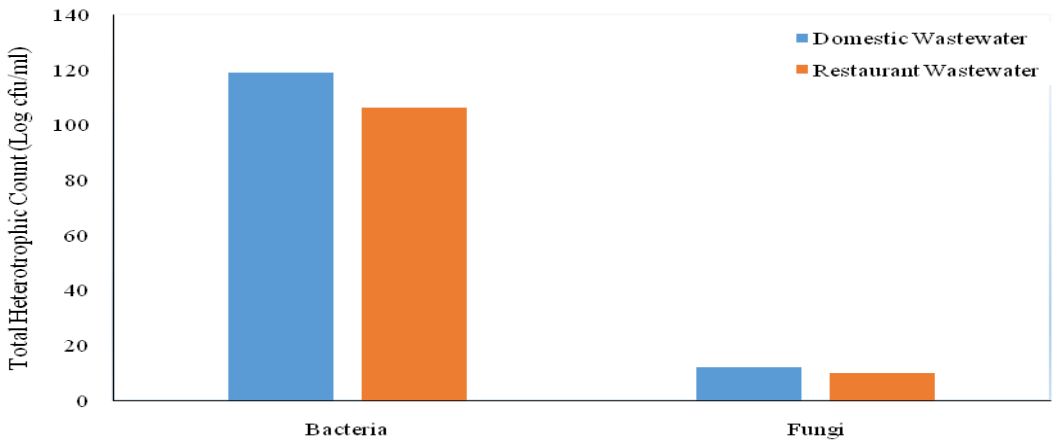

Figure 1: The total heterotrophic count for bacterial and fungal isolates.

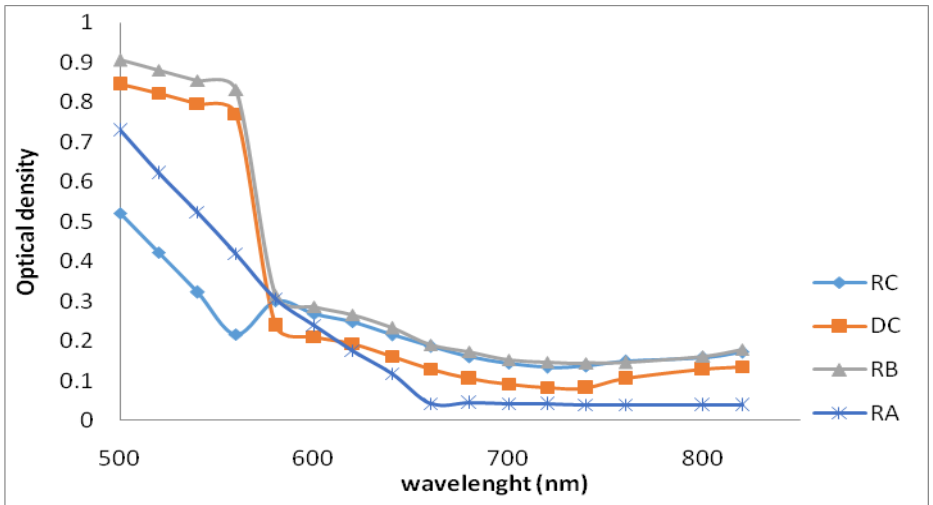

Figure 2: Wavelength selection for restaurant and domestic wastewater

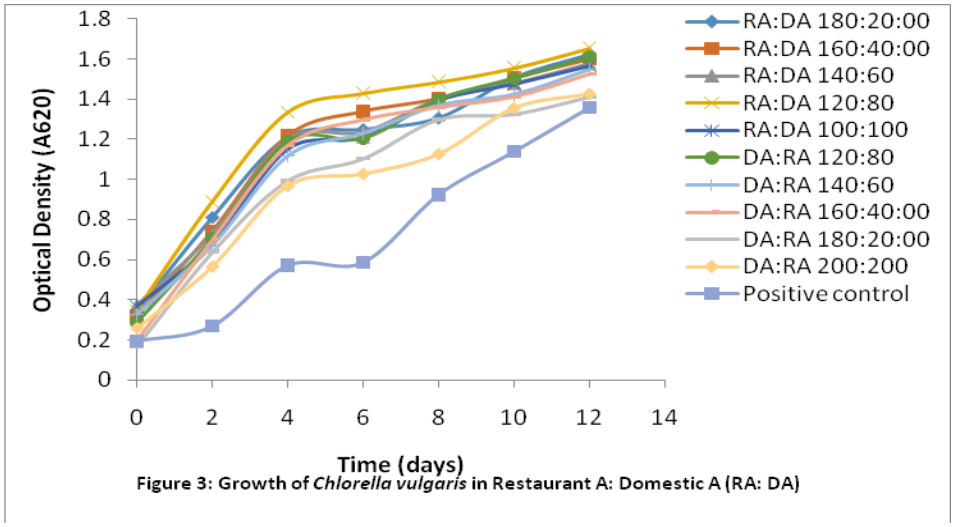

Figure 3: Growth of *Chlorella vulgaris* in Restaurant A: Domestic A (RA: DA)

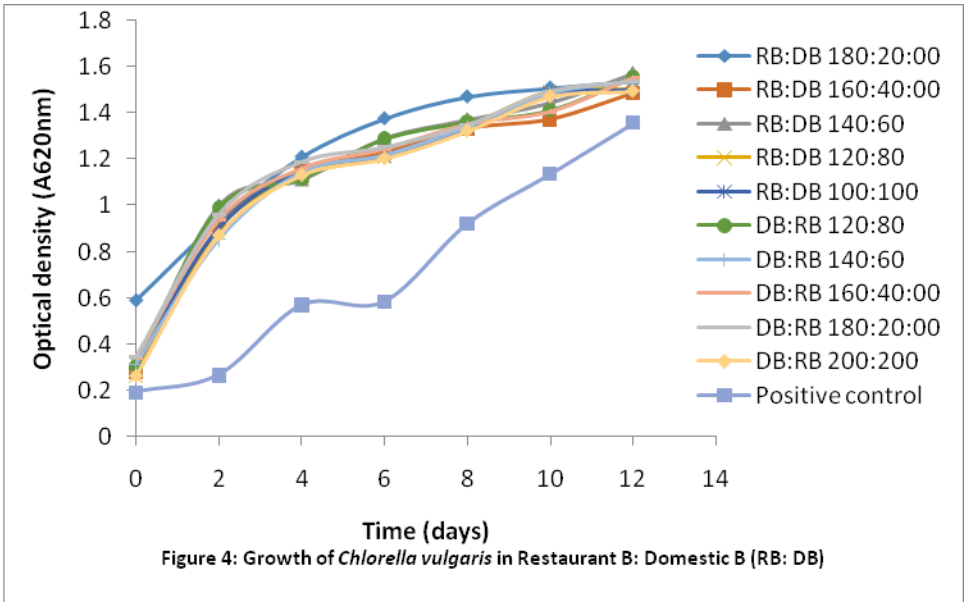

Figure 4: Growth of *Chlorella vulgaris* in Restaurant B: Domestic B (RB: DB)

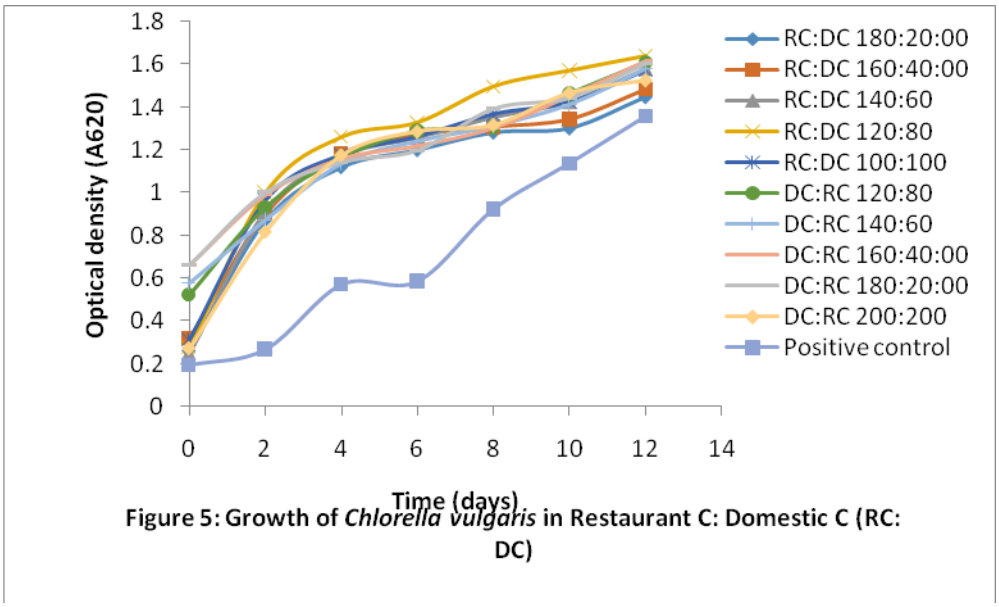

Figure 5: Growth of *Chlorella vulgaris* in Restaurant C: Domestic C (RC: DC)

The electrical conductivity for restaurant wastewater were 24.93±7.45, 271.00±6.35, 240.93±2.60 and that of domestic wastewater were 339.08±8.87, 191.03 ± 2.59 and 364.51 ± 6.50 µS/cm. These are similar with the report of Benit and Roselin (2015) who reported that the electrical conductivity was within the range of 620-2390 µS/cm. A correlation exists between high conductivity and pH. Wolfgraber *et al.*, (2012) report that presence of essential minerals can be correlated with high conductivity values. Other important factors observed were nitrate and phosphate of the restaurant wastewater, hence they act as electron acceptors or donor, thereby enhancing its quality as growth medium, the nitrate content of 19.43± 0.10, 13.60 ± 0.23, 16.52± 0.11 ppm were obtained from restaurant wastewater while domestic wastewater had values of 14.73± 0.04, 17.48 ± 0.56and 13.67 ± 0.09 which suggests that the activities in the restaurant produced rich spent waste water from washing of proteinous materials like fishes and fish product, meat and meat-products and other materials used in the restaurant services and may differ from one restaurant to another. According to Langergraber *et al.*, (2004) the nitrate and nitrogen level of a domestic or industrially generated wastewater can be useful in irrigation practices or used for flushing of the latrines to control both health and aesthetic challenges. Dissolved oxygen, Biochemical oxygen demand and chemical oxygen demand suggest the presence of organics, which

therefore suggest the presence of fecal matter or other forms of degradable organics. The chemical oxygen demand observed ranged from 176.1 -283.95 ppm and that of domestic wastewater ranged from 201.14-323.70 ppm (Table 4.2).These agree with the report of Wang *et al.*, (2008) where dissolved oxygen for domestic wastewater was 52.89, 33.62, and 54.24ppm, while the restaurant had 38.45, 62.94 and 39.36 ppm suggesting fairly high content. The lower titre reported for domestic suggest a possible blend of fecal dropping from food processing. This agrees with the findings of Osuolale and Okoh (2015) who suggested that some amount of stress is impacted on both microbial and microbial flora when the dissolved oxygen is depleted or lesser. In this study, the BOD values were in close proximity with the report of Enitan *et al.*, (2015).The total heterotrophic counts for bacterial and fungal counts for domestic wastewater were $1.19x10^6$cfu/ml and $1.2x10^4$cfu/ml while $1.06x10^6$cfu/ml and $1.0x10^4$cfu/ml for bacterial and fungal count from restaurant wastewater samples. Fig 1 shows the total heterotrophic count for bacterial and fungal isolates (Hamaidi *et al.*, 2013). The results of the wavelength and optical densities show a corresponding fall in the absorbance of the formulations as the wavelength increased (Fig.2). The wavelength increased manually from 500-820nm, the corresponding absorbance level fell from 0.9nm to 0.01nm. The process was repeated for the restaurant and domestic wastewater for increased precision in the selected wavelength for the biomass monitoring and optimization investigations. The difference in the growth pattern for the positive control and the optimal growth point is obvious in the graph presented. The growth performance of the different ratios of the restaurant and domestic wastewater portray the 120:80 which is also equivalent to the 60:40 as the best ratio for the restaurant wastewater (RW) and domestic wastewater (DW) respectively for blooming the algae. The entire growth formulation of the substrate describes the substrate ability to support the growth of the algae. The Positive control made with the four novel synthetic media had a much lower growth performance to the other ratios (Cho *et al.*, 2007). The wastewater medium has the potential ability to support the growth of microalgae.

The importance of domestic and restaurant waste water as a source of essential nutrients for the cultivation of *Chlorella* sp shown. Thus, these wastes could be channeled towards the culture of these organisms on a large scale. This process of *Chlorella* cultivation can be considered as a renewable inexpensive resource and effective waste utilization for the growth of this microalgae and these microalgae biomass can be harnessed for organic acid production.

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