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EFFECTS OF DIFFERENT STIRRING RATES ON THE *CHLORELLA VULGARIS* GROWTH FOR WASTEWATER TREATMENT SYSTEMS

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Abstract- Microalgae were found to be suitable for wastewater bioremediation. Certain parameters are capable to influence their growth, in which stirring was among these factors. In this research, three MSTPBRs R_1, R_2 and R_3, were fabricated and set at average stirring rates 50, 100 and 120rpm to assessed impact of various stirring rate for Chlorella vulgaris specie growth. After 21days of inoculation at optimum irradiance of 582.7µmol/m^2s, pH of 7.1 and using MBBM, the highest biomass concentration of 195 was obtained in R2 with 100rpm stirring rate with a corresponding estimated biomass growth rate of 0.72d^{-1}

Keywords; microalgae, aeration, stirring rates, biomass concentration, growth, MSTPBR

I. INTRODUCTION

Microalgae are considered to be the best option in wastewater treatment systems due to their ability to grow in different and varying strength of wastewater, utilizes light and carbon-dioxide (CO_2), assimilate nutrients present in the wastewater, uptake heavy metals ions in contaminated effluent solutions and provision of the renewable energy alternatives such as biodiesel, biogas, biofuels and other valuable extracts with numerous industrial applications

In order to achieve this, several biotic and abiotic factors need to be optimized to tap all the benefits associated with this novel treatment approach. These factors include light, pH, oxygen (O_2), CO_2, temperature, salinity, competition among species, dilution rate, mixing, depth, frequency of the harvesting, extracellular secretion of inhibitory substances and gas diffusion may all influence the success of algal wastewater treatment system (Agarwal *et al.,* 2019) and above all, Pedruzi *et al.*, (2020) ascertain that stirring (in other ward mechanical agitation) and aeration affects all the mentioned factors.

Mixing and aeration are significant parameters in biotechnology for wastewater and industrial effluent treatment as it facilitates organic matter degradation, that influence the respiration of microorganisms, promote CO_2 availability, provide efficient nutrients circulation, increases gaseous mass transfer rate, mitigation of stratification formation, light limitation and photoinhibition (Agarwal *et al.,* 2019; Mohammed *et al.,* 2014a; Pedruzi *et al.,* 2020)

Some credits were associated with the provision of efficient mixing such as uniform exposure of cells to the light source(s), enhancing the uptake of CO_2, and releasing, thereby reducing the rate of greenhouse gases emissions and guarding against global warming (García *et al.,* 2017). Xie *et al.,* (2017) reported that for algal cultivation, mixing not only beneficial to the CO_2 mass transfer but settling prevention as well as homogeneous mixed condition maintenance of the suspended algal cells (de la Noue *et al.,* 1984) and also cell wall destruction and introduction of modified DNA to the cells were achieved through agitation in presence of glass beads (Fu and Wang, 2011)

Stirring play vital role in maintenance of cell suspension to mitigate gradient zone stratification, nutrients distribution and enhances gas exchange (Pedruzi *et al.,* 2020). It also eliminate problems associated with provision of light like light limitation by self-shading as well as photoinhibition (Mohammed *et al.,* 2014b). Open systems such as raceways were associated with paddle wheel to achieve a thorough mixing (Pathak *et al.,* 2019; Priyadarshani *et al.,* 2014) while in a closed systems, the parameters like gas transfer, mixing and lighting were more controlled

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systematically than the open systems and achieve higher treatment efficiencies.

In the aerated systems, air is introduced to the culture usually by the a pump connected with small diameter tubes (Komolafe *et al.,* 2013) at the bottom of the reactor or container to allow mixing to prevent sedimentation, increases gas exchange and evenly distributed media, while mechanical agitation was usually provided by connecting electrical motors with stirrer shaft rotating the starring blade or impeller. Mohammed *et al.,* (2014a) achieved stirring through the use of overhead mechanical stirrer centrally provided at about 100±rpm.

The rate of growth for photosynthetic microorganisms increases with an increment of agitation until an optimum stirring rate was attained which is specie dependant (Sobczuk *et al.,* 2006) and beyond this, a sharp decrease in growth was noticed as a result of higher turbulence that damage the cells as some algal species have fragile cell wall, filamentous or mobile that can be susceptible to physical stress (Sobczuk *et al.,* 2006). Thus, a minimum possible required stirring rate should be provided in order to promote the biomass growth and subsequent extraction of valuable materials from it (Pedruzi *et al*., 2020)

Some researchers tried to differentiate the impact of aeration by the use of pumps and agitation by mechanical stirrer. Benítez *et al.,* (2018) reported that a relatively higher ammonium removal efficiency was achieved in aerated than in agitated culture by microalgal specie in PBRs, while a much greater phosphate was removed through agitation and aeration. A complete nitrification process was attained in the absence of aeration as reported by some researchers to support the idea of stirring is more important than aeration in a treatment system

Priyadarshani *et al.,* (2014) shows an increase in the growth rate and corresponding protein content in cultures supplied with aeration than those without aeration and also a proportional increase in biomass growth was reported with increment of mechanical stirring up to the optimum stirring rate and beyond that, a decrease in biomass concentration is observed due to damage of cells as a result of rapture by high gas bubbles at culture surface (Sobczuk *et al.,* 2006) and this problem is specie dependant especially those lacking rigid cell walls. Contrarily, Mustafa *et al.,* (2019) ascertained that agitation hinders the growth of L *casai* grown in pomegranate juice and biomass growth, lactic acid and cell viability was decreased as stirring rate increases from 0-150rpm.

However, considering the value of stirring in the biomass growth and productivity in microalgal wastewater treatment approach with consequent extraction of valuable renewable energy alternatives with contrary arguments of effects stirring rates to some particular biomass species, a thorough investigation of the impact of stirring and aeration will be needed in order to explore the highest benefit and selected the most promising algal strain for this purpose. Pedruzi *et al.,* (2020) strongly recommended that, the factors that influence the growth of microalgae should be thoroughly investigated and optimized in order to achieve a maximum biomass productivity that enhances the removal efficiencies of nutrients, heavy metals and other pollutants as well as increased extraction of biofuels at cost-effective and sustainable way

Based on the above, this research was intended to use the Modified Bold Basal Media (MBBM) composition reported by Alalayah *et al.,* (2015) to culture Chlorella vulgaris sp at about 50, 100 and 120rpm stirring speeds in a modified stirred-tank photobioreactors (MSTPBRs) initially developed by Mohammed *et al.,* (2014b); Mohammed, (2013) in order to assessed the *Chlorella v* sp. growth rate in response to the varied stirring rates and subsequent other treatment and recovery processes

II. MATERIALS AND METHODS

MSTPBR fabrication

The 22L MSTPBR centrally illumination chamber was fabricated using a transparent plexiglas material (Globe Plastic Industries; Malaysia) of 9cm in diameter and 25cm depth housing a 2cm diameter for stirrer shaft. The overall capacity of the MSTPBR was 32cmX27cm with a working volume of about 16L, which allowed about 30% headspace for degassing. Designed as semi-continuous reactor a tap was introduced at the bottom side of the MSTPBR for sample collection and mixing was provided using an overhead mechanical stirrer attached to an electric motor. Three MSTPBRs R_1, R_2 and R_3 were inoculated with 16L of culture of microalgae and BBM solution to assessed the algal growth under different stirring rate

Cultivation of algae

The *Chlorella v*. sp. was obtained from Herbarium Laboratory, Department of Plant Biology, Bayero University Kano, Nigeria and cultivated in a 1L Pyrex conical flask using Modified Bold Basal Medium (MBBM) in accordance with Alalayah *et al.,* (2015), the composition of the mixture was shown in Table 1.cultured at about 280C and

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externally illuminated using cool white fluorescent tube (CTL-EL 102), for 8/16 light/dark photoperiod for 14days. All the chemicals used were of analytical grade purchased from Shumtech

S/ No	Formula	Weigh t (g)	Distil Water (L)
1	K_2HPO_4	1.875	250
2	KH_2PO_4	4.375	250
3	$MgSO_4.7H_2O$	1.875	250
4	$NaNO_3$	6.250	250
5	$CaCl_2$.$4H_2O$	0.625	250
6	NaCl	0.625	250
7	Na_4 EDTA	5.000	100
8	KOH	3.100	100
9	$FeSO_4.7H_2O$	0.498	100
10	H_3BO_3	1.142	25
11	$MnCl_2.4H_2O$	0.058	25
12	$ZnSO_4$. $7H_2O$	0.353	25
13	$Co(NO)_2.6H_2O$	0.020	25
14	Na_2MoO_4. $2H_2O$	0.048	25

Scientific and Laboratory Supply, Kano, Nigeria

Inoculum 1
About 0.022g of immobilized *chlorella v.* was weighed on an electronic weighing balance (ADAM : PW 214) and inoculated in a MBBM in a 1L Pyrex conical flask aerated using an air pump (haning Beach: SB660, China). This inoculated culture was externally illuminated by fluorescent bulb at 8/16 light and dark photoperiod for a period of 14days

Inoculum 2
The inoculum 1 was scaled-up and cultured in MBBM as previously described by Alalayah *et al.*, (2015) in 22L MSTPBRs. The cultures in R_1, R_2 and R_3 were mixed thoroughly using an overhead mechanical stirrer at about 50, 100 and 120rpm respectively

Table 1: Composition of BBM

Alalayah *et al.*, (2015)

Illumination
The reactors R_1, R_2 and R_3 were illuminated internally from the central core made of transparent Plexiglas, providing the light radially from the centre of the MSTPBRs by 189 red light emitting diodes (LEDs) (Kehong Opto Electronics, KH – T542URC25-K, Guangdong, China) array on 9-vero boards at an optimum irradiance of about 582.7µmol/m^2sin accordance with Mohammed *et al.*, (2014b) from the central core to illuminate each of the 22L reactors

Experimental Set-up
The MSTBRs (R_1, R_2 and R_3) as shown in Fig.1, were inoculated with culture medium (algal culture + MBBM) to a working volume of 16L, illuminated to about an optimum irradiance of 582.7µmol/m^2s, at 12/12 light-dark cycles.

The stirrers were set at an average speed of 50, 100 and 120rpm for R_1, R_2 and R_3 respectively.

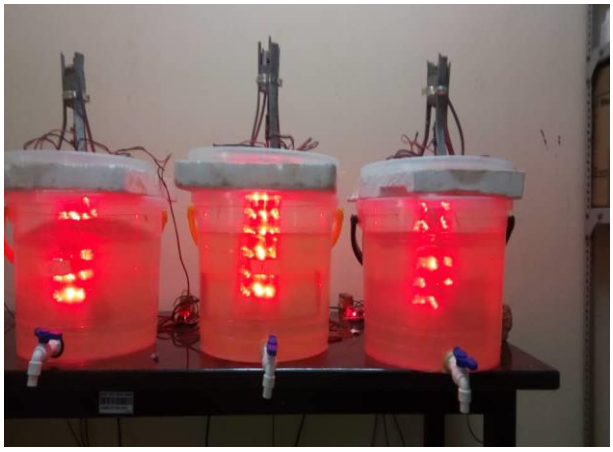

Fig. 1 Modified Stirred-Tank Photobioreactors MSTPBRs set-up

Aluminium foil was used to wrap the photobioreactors in order to minimize loss of light. The samples were collected at 2days interval for total suspended solids (TSS) analysis in accordance with Sun *et al.*, (2019) in order to estimate the microalgal growth under the influence of varied stirring rate using Calorimeter, Hach: DR/890 series. The MBBM media was used as control and the pH of the culture was maintained at 7.2 using a pH meter and adjusted by hydrochloric HCl and sodium chloride (Nacl)

Estimation of *Chlorella v.* Concentration
Both sample cell and cell compartment of the Colorimeter (Hach: DR/890) were cleaned with tissue paper and then, the programme number for TSS as 94 was selected and the media reagent solution was used to zero the instrument in which 25ml of the MBBM solution as blank was placed in

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the sample cell and with diamond-shaped marker toward the keypads, which was then rotated until it was aligned properly in the slot. The Colorimeter cap was used to cover the test compartment in order to shield against the external stray light and contributed to the accuracy of the result. The READ key was press and this was stored as zero reading and the Colorimeter was then ready for subsequent TSS measurements. Three samples from R_1, R_2 and R_3 was prepared and shaken and then the placed in the sample cell compartment one after another for TSS as initially done for blank solution and the displayed results was recorded in mg/L.

III. RESULTS

The MBBM media was used as control to zero the calorimeter for the estimation of algal concentration as TSS and the initial TSS value after 2weeks in inoculum 1, was found to be 139mg/L and this was subsequently diluted, up-scaled and cultured as inoculum 2, in the three reactors with following concentrations at interval of 3days in table 2.

Table 2: *Chlorella v.* growth under different stirring rate

Test No.	Algal Concentration (mg/L)		
	R1(50rpm)	R2(100rpm)	R3(120rpm)
Test 1	5	1	2
Test 2	20	26	9
Test 3	28	30	40
Test 4	33	34	49
Test 5	50	48	70
Test 6	65	105	86
Test 7	90	195	122

From the result, it can be understood that in R1 with 50rpm stirring rate, the concentration of the biomass from an initial concentration of 5mg/L to maximum attained as 90mg/L, within the first three weeks of chlorella vulgaris cultivation. The growth increases gently up to about 2 weeks of cultivation and after that a sharp increment was noticed. In R2, a more rigorous initial growth was obtained between first cultured concentrations of about 1mg/L to as high as 26mg/L within 3days which increase rapidly up 195mg/L for the span of 3weeks. R3 shows a moderate biomass concentration of until it reaches 9mg/L, after then a higher specific growth was noticed especially after the first week of cultivation which then maintained a moderate biomass growth within the reactor by the end of week three of the culture a concentration of 122ml/L was observes.

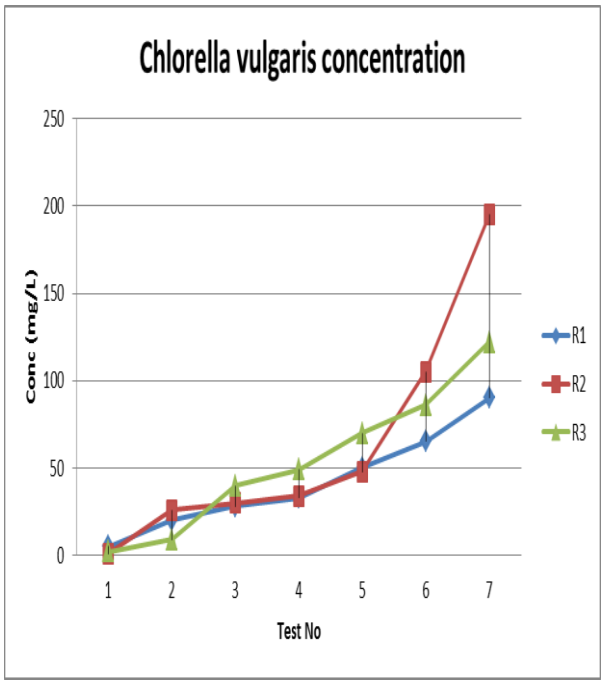

Fig. 2. Chlorella vulgaris growth within three weeks of cultivation

About O.09, 0.72 and 0.14d^{-1} growth rate was estimated in accordance with the approach reported by Arnas, (2014) and detailed interrelationship between the biomass concentrations of R_1, R_2 and R_3 with their corresponding stirring rates could be clearly seen from Fig. 2.

IV. DISCUSSIONS

Growth rate is one of the most crucial factors in understanding the level of adaptation to environmental conditions and succession of particular specie in the treatment medium or ecology. Extremely higher initial growth rates was obtained especially in R_2 and R_3 of 0.72 and 0.14d^{-1} respectively, which were associated with higher stirring rates compared to that of R1 with low stirring rate. These growth rates was significantly higher than those reported by many researcher, Mohammed *et al.*, (2014b) reported a relatively lower biomass growth rate and productivity of 0.109 d^{-1} and 0.034$gL^{-1}d^{-1}$ despite the enhancement of CO_2 supplementation to the cultures.

Priyadarshani *et al.*, (2014) argued that the aeration and light not always proportional to the increasing the biomass growth as the specific growth rates under these conditions had no significant difference at 5% confidence level. Mohammed *et al.*, (2014b) hold a similar view on this as reported that the biomass growth was not always directly proportional to the irradiance provided to the culture and tested biomass productivity at irradiance of 429.9, 582.7 and 730.8 µmol/m^2s in a

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the highest growth was observed in the reactor with medium irradiance of 582.7 µmol/m^2s. This can also be observed from current research in Fig.2, where despite the employment of various stirring rates of 50, 100 and 120rpm in the three MSTPBRs, there is no significant difference in the reactors especially within the first 10 days of the cultivation

During the first 12 days of the cultivation, none of the three culture growth above 50mg/L, and after then within the subsequent 9days the biomass growth reached 195mg/L. de la Noue *et al.,* (1984) explained a related scenario in which during the first 3days of cultivation, the algal production continuous at about uniform rates in both cultures and then the biomass production seizes in the stirred cultures as 80-100mg/L be significant as per as algal biomass growth and productivity is concerned was observed in the first 10days, while in aerated cultures increases daily by about 100mg/L and after 10days of cultivation the biomass production reaches 700-750mg/L which was found to

The biomass growth and productivity of the photosynthetic microorganisms increases with increment of turbulence induced by mechanical agitation (Pedruzi *et al.,* 2020), this was noticed from Fig. 2 especially after the first 14days of the culture as the biomass concentrations in the R_2 and R_3 with their corresponding stirring rates of 100 and 120rpm respectively start to show significant increase than the R_1 with low stirring rates of 50rpm. This in line with the finding by de la Noue *et al.,* (1984) that during first eight days of the cultivation, the biomass production seemed to be unaffected to the aeration rates and after that a considerable increase in biomass production was observed

The biomass concentration of R2 overtakes that of the R3 after about first 16days of cultivation and attained a higher productivity of about 195mg/L with respect to that of 122mg/L of R3 despite having higher stirring rate of 120rpm, within the range of last 5days of the test. This was found to be more interesting as initially perceived that the highest biomass productivities and growth rates should be directly proportional to the rate of stirring provided and that the other growth parameters like illumination culture medium, working volume and pH were kept constant.

Contrary to the above, increase in biomass production was reported by corresponding increase in the rate of stirring until a certain optimum level was reached and after that, a sharp declined of biomass concentration was observed (Pedruzi *et al.,* 2020). Sobczuk *et al.,* (2006) submitted that, the agitation by stirrer effectively improve the biomass production and increase in stirring rates up to the 350rpm that correspond to the stirrer's tip speed of 1.4m/s shows no sign of damage to the algal cells, but the damage of the hard protective cell wall of the eukaryotes was attributed not to stirring but sparging of gas at a speed above 1.5m/s of the stirrer's blades. As such a moderate stirring rate of about 120rpm should however, provide an optimum biomass concentration without hindrance, since it was not even near the maximum identified stirring rate of 350rpm that contribute to the decline in biomass growth and productivity.

However, despite the higher initial growth rates of the culture with about 15-fold, 25-fold and 7-fold of growth at R_1, R_2 and R_3 respectively, a more closely monitoring of the treatment system will be provided in order to assessed the influence of stirring rates of 100rpm and 120rpm for R2 and R3 with their correspondence variations of biomass productivities especially with regards to the longer treatment period and the rate of biomass produced with its subsequent use for bioremediation process for sustainable wastewater treatment system at cost effective and production of energy alternatives such as biogas, biodiesel and other valuable materials.

V. CONCLUSION

Based on results of the investigation, it could be concluded that under optimum conditions of pH, illumination and growth medium inoculated for 21days, the Chlorella vulgaris concentration in R_1, R_2 and R_3 to be 90, 195 and 122mg/L at an average stirring rates of 50, 100 and 120rpm respectively. Also a higher initial growth rates for R_1, R_2 and R_3 was estimated to be 0.09, 0.72 and 0.14d^{-1} respectively.

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