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Treatment of Municipal Wastewater using Oxygenic Photo granules (OPGs)

[1] Arya Chandran J, [2] K. Mophin Kani, [3] Anu N
[1][2][3] Environmental Engineering & Management, Department of Civil Engineering
UKF College of Engineering and Technology, Parippally, Kollam, Kerala

Abstract: **Wastewater treatment methods are energy intensive. Although, activated sludge process is effective in removing organic matter but it consume large amount of energy, particularly in the aeration process. However, wastewater itself is a source of energy, if we capture this energy from wastewater, wastewater treatment might become an energy producer rather than a consumer. Compared to activated sludge process, oxygenic photogranules have potential to treat wastewater without external aeration. In addition it produces algal biomass, which is favorable to use for bio-fuel production. Hence wastewater treatment system with oxygenic photogranules reduces energy consumption and produce energy feed stock. This study was mainly focused on the synthesis of oxygenic photogranules from activated sludge in the presence of sunlight under unagitated condition for 42 days and then conducted the treatment of real municipal wastewater with these oxygenic photogranules. OPGs can remove COD and nitrogenous compounds without external aeration. In the present study, COD and nutrients like nitrogen and phosphate removal efficiencies obtained was 98.5%, 97.09% and 99.18% respectively. The OPG process eliminate the need of external mechanical aeration, which is the most energy intensive part of wastewater treatment processes, and allow the recovery of chemical energy from wastewater in the form of bioenergy feedstock. OPGs are easily harvestable biomass, overcoming the main challenges of algal based wastewater treatment technologies. In this study, efficiency of OPG in treating real municipal wastewater without external aeration was discovered.**

Keywords: Biogranules, Municipal wastewater, Oxygenic photogranules, Wastewater treatment

INTRODUCTION:

In the last decades, the generation of wastewater has been rapidly increasing with population growth, urbanization and industrialization, and the amounts produced are already far beyond the self-cleaning limit of natural aquatic systems. Thus, it is essential to treat such wastewater before discharge to protect human population and ecosystem, as well as to improve our environmental quality (Prasse C et al, 2015).Wastewater can be defined as water from sewage pipes and runoff over ground in which the water has become contaminated due to anthropogenic activity. Urbanization and industrialization influence the extent of pollution. In particular biological treatments play an important role, being responsible for removing more than one half of the total organic pollutants in wastewater. They are also considered environmentally friendly technologies owing to less chemical use, and energy input as well as renewable energy generation under certain conditions (Chong S et al, 2012).In biological treatment units, it is better to retain dense mass to ensure the effectiveness of treatment and increase economic feasibility. Especially, it can directly determine the success of operation when slow growing microbial species perform main function. Instead of using extra equipments, microbial granulation can be applied as an alternative method to attain high treatment efficiency. The conventional activated sludge system has the most important in wastewater treatment plants (WWTP).

A conventional activated sludge system generally consist of an aeration tank where air enhances the biological processes between microorganisms, a settling tank, or clarifier that separate clear effluent from sludge and a disinfection tank to remove pathogenic microorganisms further. The major disadvantage of conventional activated sludge system is the requirement of large footprint in response to the long settling phase and large number of units for treatment (de Kreuk, 2006). The granules in this system do not settle rapidly due to slow growth rate of nitrifying microorganisms. Although the activated sludge system in WWTPs is found to be most common in today's industry. Water and Wastewater treatments are energy intensive process. Processes included in water and wastewater treatment consume large amount of energy that represent an important portion of worldwide energy consumption (Stillwell et al., 2010). Activated sludge process is widely used for the treatment of wastewater but it consumes 60% of energy available at the treatment plant. Because of this high energy consumption required for aeration limits its potential to use as a sustainable treatment method (Bouhend et al., 2016).

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Untreated wastewater is a significant energy resource; about 6-8MJ of chemical energy is present in 1 m3 of typical wastewater, which means that wastewater holds more than three times the energy used to treat it. Therefore we can capture this potential energy from wastewater; the wastewater treatment process might become an energy producer rather than a consumer. In engineered applications, the oxygenic nature of some cyanobacteria and their symbiosis with other bacteria species make them alternative for biological treatment. However separation of cyanobacteria has been a major barrier to implementation. A potential solution to this issue is the recently cultivated, oxygenic photogranules produced from photo-illuminated activated sludge (Stauch White et al., 2017). Compared to activated sludge process oxygenic photo granules have potential to treat wastewater without external aeration (Christenson and Sims, 2011). In addition it produces algal biomass which is favorable to use for bio-fuel production. Hence wastewater treatment system with oxygenic photogranules reduces energy consumption and produce energy feed stock (Bouhend et al., 2016).

OPGs are the most recent addition to the family of bio-granules for biotechnological applications. The formation of these granules was first documented in unagitated, sealed vials exposed to natural light by Park and Dolan. For biotechnological applications like wastewater treatment, OPGs possess a number of properties that make them potential candidates for alternatives to the costly activated sludge process. Many successful OPGs cultivation have been performed under a variety of conditions and from a variety of municipal waste streams around the world. The oxygenic photogranule is a new algal-sludge granule wastewater treatment process based upon the photo granulation of activated sludge in to OPGs. During maturation, granules increase their net density via compaction, i.e., the loss of filamentous cells, and through the precipitation of calcite in the interior of granules.

Oxygenic photogranules are naturally formed and composed of bacteria and algae. It is a dense, spherical and easily settleable granular bio-film of heterotrophs, autotrophs and microalgae (Milferstedt et al. in review). The bacteria degrade organic matter using oxygen produced by algae. In addition algae utilize the carbon dioxide produced from the organic matter degradation for photosynthesis. Oxygenic photogranules work without external aeration and consume more carbon dioxide and thus reducing greenhouse gas emissions and making the system carbon negative. Oxygenic bio-granules reduce sludge production and have the potential to bring energy savings for municipal wastewater treatment (Chul Park et al., 2017). Oxygenic photogranules remove chemical oxygen demand and nitrogenous compounds without external aeration. Without external aeration, wastewater facilities could save 25-60% of operating cost of municipal wastewater treatment (Abouhend et al. 2016; Milferstedt et al. in review).Applications of oxygenic photogranules include enhanced nitrogen removal, removal of heavy metals, reduction of wastewater treatment carbon footprint; bio-energy feed stock generation and nutrient treatment and removal at wastewater treatment plant with minimum energy investment (Chul Park et al., 2017).

This thesis was particularly focused on the aspect of wastewater treatment by means of oxygenic photogranules technology. This study was hypothesized that the production of Oxygenic photogranules are important because conventional activated sludge process consume more time and energy for municipal wastewater treatment and Oxygenic photogranules have ability to treat municipal wastewater.

MATERIALS AND METHODS

2.1 OPGs Cultivation

Activated sludge was directly sampled from the aeration basin of the Sewage Treatment Plant, Muttathara, Trivandrum. Upon returning to the lab, 7 mL sludge were pipetted into 15 mL vials and then closed with a plastic cap, leaving head space. During granulation, the biomass remained in these vials. The closed vials therefore be considered closed ecosystem where only energy in the form of light enters. During granulation, the vials were neither moved nor shaken or agitated in another form. Vials were illuminated under sunlight and fluorescent lights of 20W, 24 hours per day at room temperature for 42 days. The biomass in one vial yields precisely one photogranule that is typically situated at the bottom of the vial. At day 42, the cultivation was considered as complete. To determine the success of granulation, shake test was performed by using three firm vertical shake and then observing the vial contents. When a granule remained without particulates in the bulk liquid, granulation was considered as successful.

2.2 OPG Characteristics Identification

2.2.1 Microscopy

Microscopy was performed on a Radical RMH-4B (220V, 50Hz) light microscope by sectioning granules in a petri dish with a razor blade and then placing sampled sections on a microscope slide. At least three sections from each selected granule were inspected.

2.2.2 Scanning Electron Microscopy

The OPG characterization such as SEM was done at Sophisticated Instrumentation and Computation Centre, University of Kerala, Kollam.Samples for SEM were fixed in an unbuffered solution of 1% glutaraldehyde added to each sample and gently agitated for approximately 3-4h. Samples were removed from the fixative into petri dish and manually cross-sectioned under a microscope to visually determine the maximum diameter. Next, samples were washed three times for 10 min each in phosphate buffer solution. Samples were post fixed in 1% osmium tetroxide in the same phosphate buffer for 1.5h on a rotator at room temperature, and then washed for 15 min. Following these steps, samples were washed three times for 40 min in Milli-Q water. Each sample was dehydrated through graded ethanol series. Samples were stored overnight in 70% ethanol at 200 C. The next day, the fixed samples were warmed to room temperature and ethanol dehydration was continued. The samples were dried using the tertiary butanol method. Samples were subsequently imaged using EVO18 SEM operated at 10 kV.

2.2.3 Settling Velocity

Sample used for settling velocity determination was placed inside a 1L capacity measuring cylinder which was filled with water upto 800 mL mark. Then note the time taken by the sample to reach the bottom of cylinder or to a predetermined mark. Also note the distance between the initial and final position of the sample. Then Settling Velocity was calculated using the following equation:

Settling Velocity = (distance)/ Time= D/t (2.1)

2.2.4 Porosity

Sample used for porosity determination was first dried in an oven at 1050C for 2 hours. After that sample was taken out from the oven and note the weight of dried sample. Then the dry granule was placed inside a container which was filled with water. Weight of granule inside the container was measured periodically until the weight remaining constant (that means sample was completely saturated). Difference between the weight of dry granule and final weight indicates mass of water inside granule. From the mass of water and density of water, volume of water was calculated. Volume of water in the granule was equivalent to volume of voids. Then Porosity was calculated using the following equation:

Porosity = Volume of voids/ Total volume of sample= VV/V (2.2)

2.2.5 Density

For the determination of density of granules, first weight of granule was noted. Volume of granule was determined by placing the granule inside a container which was filled with water completely. The amount of water displaced from the container due to the granule was collected and measured. That value indicates volume of granule. Density of granule was calculated using the equation:

Density = Mass of granule/ Volume of granule =M/V (2.3)

2.3 Municipal Wastewater Collection

The municipal wastewater used in this study was obtained from the Collection tank of Sewage Treatment Plant, Muttathara, Trivandrum. It was collected in a plastic bottle and should keep in a refrigerator at a temperature of 4oc. The standard method for examination of water and Wastewater was followed for sample Collection and Storage (APHA, 2012).

2.4 Reactor Setup

Sequencing Batch Reactor made of acrylic material has been used in the present study. The reactor was 100 cm in length, 16 cm width and 15 cm height. Total length of the reactor was divided into six compartments each having 15.3 cm length, 15cm height and 16cm width with a working volume of 3litres. The reactors consist of stirrers in order to prevent the settling of the granules. It was installed in ambient room temperature without any temperature regulator facility. The reactors were filled with 1L sewage. The reactors were seeded with pre-cultured mature OPGs having varying dosages of 6, 8, 10, 12 and 14g/L and operated 1 hour cycles per day. Each cycle consist of 5minutes influent feeding, 40 minutes mixing, 10 minutes settling and 5 minutes for decanting. After each cycle supernatant was collected and chemical analysis was performed. Figure 2.1 shows the sequencing batch reactor set up used in this study.

Fig 2.1Sequencing Batch Reactor

2.5 Chemical Analysis

The standard method for examination of water and wastewater was followed for sample collection and chemical analysis (APHA, 2012).

III. RESULTS AND DISCUSSIONS

3.1 Initial Characteristics Of Municipal Wastewater

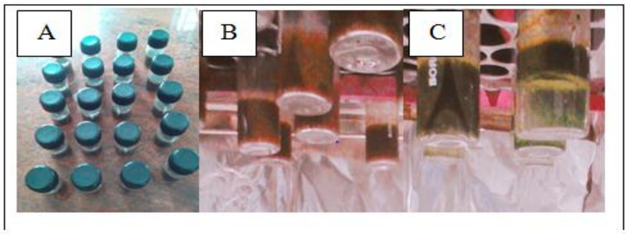
The initial characteristics of municipal wastewater were carried out. Table 3.1 shows the initial characteristics of Municipal Wastewater (sewage) collected from the Sewage Treatment Plant, Muttathara, Trivandrum.

Table 3.1 Initial characteristics of MWW

Sl. No.	Parameter	Initial Value
1	COD (mg/L)	536
2	Nitrate (mg/L)	7.23
3	Phosphate (mg/L)	13.47
4	TSS (mg/L)	340
5	SVI (ml/g)	55

3.2 Photogranulation Progression

It was found that under a batch static condition with light, typical activated sludge mixed liquor changes to a dense spherical aggregate of algae- sludge biomass. Unlike the flocs or aggregates in activated sludge, seed OPGs formed were dense, pellet like aggregates with a diameter of 5-12mm.OPGs generation in the reactor was consistent and gradual process which was checked out through many stages of developing and maturation that was identified by the change in the shape, structure and sphericity of OPG besides the increase in OPG numbers, diameters and TSS concentration. Using microscopic analysis we found that granules were composed of thick layer of filamentous microalgae, and inner part composed of aggregates of green algae and heterotrophic sludge bacteria. Figure 3.1 shows the progress of photogranulation process.



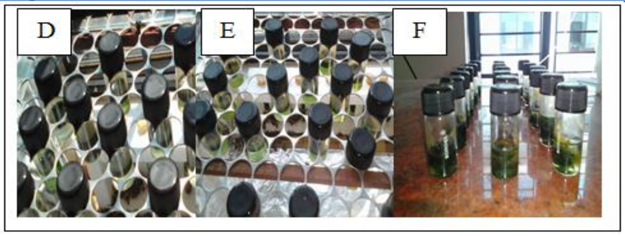


Fig 3.1: Development of OPGs A) Day 0 B) Day 0 C) After 7 days D) After 15 day E) After 33 days F) After 42 days

3.3 Characteristics Of Oxygenic Photogranules

3.3.1 Properties of Oxygenic Photogranules

Table 3.2 shows the properties of OPGs developed from activated sludge under static condition with light. Properties of OPGs were determined by performing various experiments in the laboratory including SEM analysis and algal identification test.

Table 3.2 Properties of Oxygenic Photogranules

Sl. No	Characteristics	Value
1	Size (mm)	5-12
2	Settling Velocity (m/hr)	30
3	Density (kg/m^3)	1418
4	Porosity (%)	15.55

4.3.2 SEM Analysis Result

Usually SEM is capable of producing precise details of the sample less than 1 nm in size. Fig 3.2 indicates the result obtained from the SEM analysis of oxygenic photogranules at varying magnifications. SEM image reveals that granules were composed of thick outer layer of filamentous microalgae, and inner part consisted of aggregates of green algae and heterotropic sludge bacteria.

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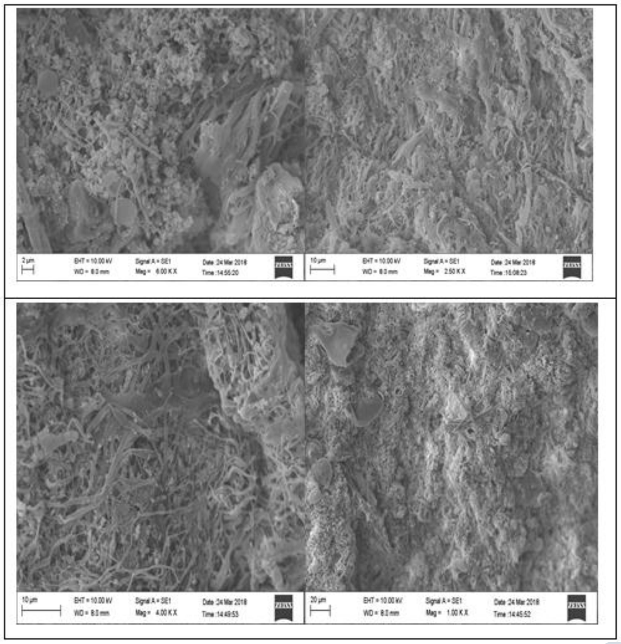

Fig 3.2 SEM images of oxygenic photogranules. Scale bars for panels are A) 2µm B, C) 10 µm D) 20 µm

3.4 OPG Reactor Performance

The SBR seeded with the mature OPGs were operated without external aeration, which is currently the most energy intensive part of the wastewater treatment. It shows high efficiency in the removal of COD and nutrients. OPGs achieve COD removal efficiency higher than 90% and average effluent COD was maintained below 10mg/L, meeting regulatory permits. The nitrogen and phosphate data also showed that OPGs system achieved better efficiency in removal of nitrate and phosphate. Nitrate removal efficiency obtained was 96% and phosphate removal efficiency was above 95%. The value of effluent nitrate and phosphate were almost equal to zero. Figure 3.3 shows the removal efficiency of COD using OPG reactor treating real municipal wastewater.

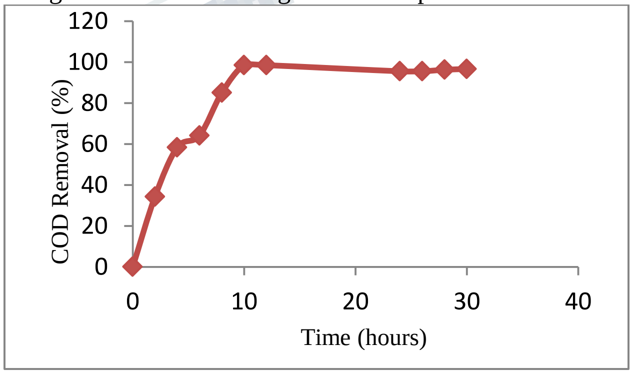

Fig 3.3 Removal efficiency of COD by using bench scale OPG reactors treating real wastewater

3.5 Changes in Biomass Concentration during Reactor Performance

During reactor operation, it was noted that there was a gradual change in the biomass concentration. After starting the treatment process, the size of seeded OPGs were changed because microorganisms in the OPGs were consume organic matter present in the real sewage and start increasing its population inside the granule there by increasing its size. Change in biomass concentration was determined by taking the weight of granules after the completion of each cycle, which means at the end of two hour time periods. Figure 3.4 represent the change in biomass concentration in terms of weight with respect to time interval.

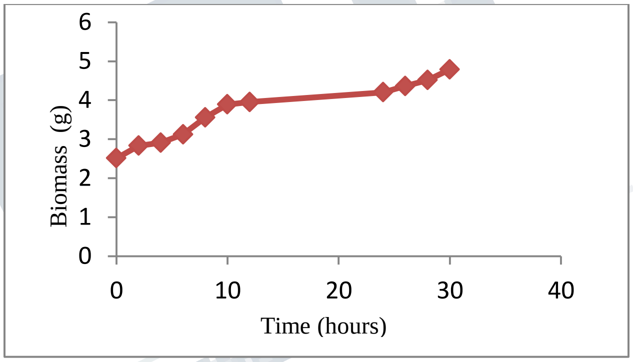

Fig 3.4 Change in Biomass concentration during treatment

3.6 Hydraulic Retention Time (HRT) for OPG Treatment

Table 3.3 shows the hydraulic retention time for treating real municipal wastewater using bench scale reactors seeded with mature OPGs. The time was varied from 2-30 hours with a time interval of 2 hours. For the determination of hydraulic retention time for treatment process, the speed of mixing was kept at 5rpm and dosage was kept 10g/L.The greatest decrease was seen at the time of 10 hour which reduced the COD from 536 mg/L to 8 mg/L. This value meeting the regulatory limit. At this time the efficiency of the OPGs in removing TSS and nutrients were also high, reducing TSS from 340 to 30mg/L and nitrate from 7.23 to 0.23 mg/L. Fig 3.5 and 3.6 shows the effect of hydraulic retention time for the treatment of municipal wastewater using oxygenic photogranules in the form of removal efficiencies.

Table 3.3 Hydraulic retention time for treating real municipal wastewater

Parameter/ Time (hrs)	COD (mg/L)	TSS (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
0	536	340	7.23	13.47

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2	352	300	5.25	5.49
4	223	260	4.15	3.61
6	192	136	2.35	1.36
8	80	84	1.87	0.79
10	8	30	0.23	0.11
12	8	29	0.21	0.12
24	24	29	0.24	0.14
26	24	30	0.24	0.21
28	20	30	0.21	0.19
30	18	29	0.16	0.21

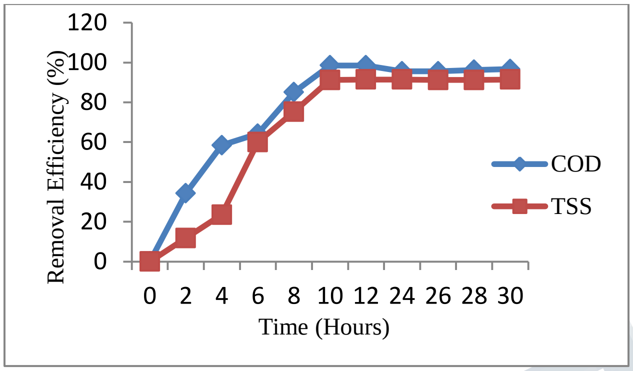

Fig 3.5 Effect of HRT in the removal of COD & TSS during sewage treatment

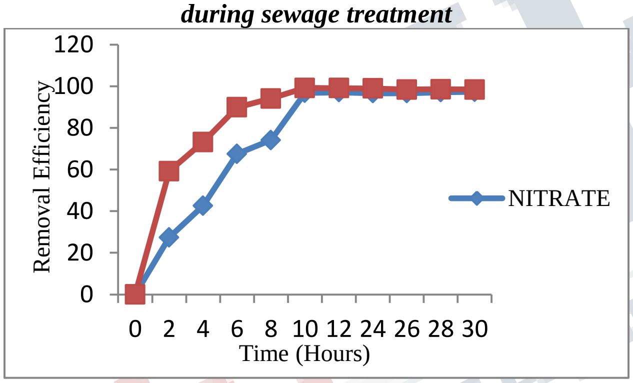

Fig 3.6 Effect of HRT in the removal of Nitrate & Phosphate during sewage treatment

3.7 Optimum OPGs Dosage for Sewage Treatment

Table 3.4 shows the optimum OPGs dosage for treating real municipal wastewater using bench scale reactors seeded with varying dosage of mature OPGs. The dosages were varied from 6-14g/L with 2g/L interval. For the determination of effect of dosage on treatment the speed of mixing was kept at 5rpm and time was kept at 10 hour interval.

Table 3.4 Dosage of OPGs for treating sewage

Parameter (mg/L)	Control	6g/L	8g/L	10g/L	12g/L	14g/L
COD	536	192	160	8	16	24
TSS	340	150	98	30	30	28
NO_3^-	7.23	3.48	2.21	0.21	0.22	0.21
PO_4^{3-}	13.47	8.39	3.31	0.11	0.14	0.13

The greatest decrease was seen at the dose of 10g/L of real wastewater which reduced the COD from 536 mg/L to 8 mg/L. This value meeting the regulatory permit. At this dosage the efficiency of the OPGs in removing TSS and nutrients were also high, reducing TSS from 340 to 30 mg/L and nitrate from 7.23 to 0.21 mg/L and phosphate from 13.47 to 0.11 mg/L respectively. Fig 3.7 & 3.8 shows the Dosage of OPGs in treating sewage.

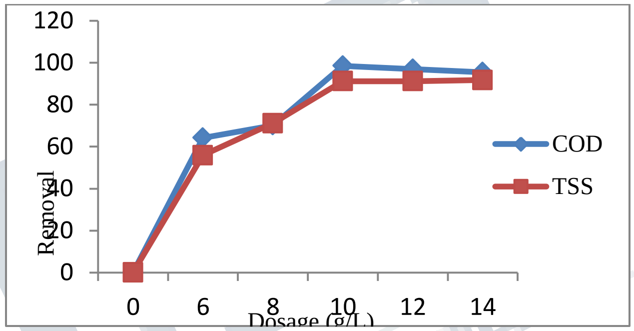

Fig 3.7 Effect of dosage in the removal of COD & TSS during sewage treatment

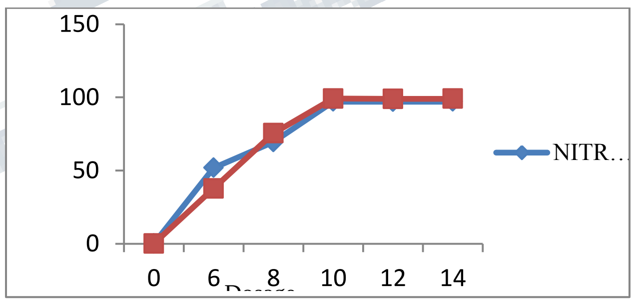

Fig 3.8 Effect of dosage in the removal of Nitrate & Phosphate during sewage treatment

3.8 Solid Retention Time (SRT)

OPGs biomass was wasted from the reactor on a daily basis and the volume of harvested biomass were used for the determination of solid retention time. In this study, solid retention time was calculated using the following equation:

$$SRT= \{\sum_i X_i V_i\}/ \{Q_x X_x \} \quad (3.1)$$

Where V_i = individual reactor volume (m^3)
X_i = MLSS in each reactor (mg/L)
Q_X = excess biosolids removal rate (m^3/d)
X_X = MLSS in the excess biosolids flow (mg/L)

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From the treatment solid retention time for treating municipal wastewater using oxygenic photogranules obtained was 40 days. After 40 days of operation, a portion of OPGs are removed and fresh OPGs are added to the system.

3.9 OPG Biomass Settleability

Harvesting of biomass is main challenges of algae wastewater treatment. The settling performance determines the settling time required before decanting of clean effluent. Achieving a short settling time by generating a rapidly settling biomass leads to significant improvement in the treatment process efficiency, particularly in the SBR operation. OPGs settled very quickly with an average settling velocity of 30 m/hr, providing easily harvestable biomass. This effective biomass settling makes biomass harvesting and recycling very easy, overcoming the main challenges associated with algae based wastewater treatment.

3.10 Comparative Assessment- Activated Sludge Process & Oxygenic Photogranules

Table 3.5 shows the results obtained after treating real municipal wastewater using OPGs without aeration and Activated sludge process with external aeration.

Table 3.5 Parameter of sewage after ASP & OPG

Parameter	Initial Value	ASP	OPG process
COD (mg/L)	536	16	8
TSS (mg/L)	340	40	30
Nitrate (mg/L)	7.23	1.12	0.21
Phosphate(mg/L)	13.47	3.75	0.11

From the table 3.5, it is clear that oxygenic photogranule process is better than activated sludge process for treating municipal wastewater especially for the removal of chemical oxygen demand and nutrients such as nitrate and phosphate. Sludge produced after treatment can be used for generating bioenergy feed stock e.g. biodiesel, fertilizer, bioethanol, and biomass that can be burned to produce heat and electricity. Activated sludge process require external aeration for treating sewage, hence it consume large amount of energy in the form of electricity. But OPG treats sewage effectively without external aeration. Therefore, OPG process is a cost effective method for treatment of sewage. Table 3.6 shows the efficiency of ASP and OPG process in treating sewage.

Table 3.6 Efficiency of ASP & OPG Process in sewage

Parameter	COD (mg/L)	TSS (mg/L)	Nitrate (mg/L)	Phosphate (mg/L)
Initial value	536	340	7.23	13.47
ASP	16	40	1.12	3.75
OPG Process	8	30	0.21	0.11
Efficiency of ASP (%)	97	88.23	84.5	72.16
Efficiency of OPG process (%)	98.5	91.18	97.09	99.18

From table 3.8, it is clear that OPG process is better than activated sludge process for MWW treatment. OPG obtain better COD and nutrients removal without external aeration as compared to activated sludge process. Fig 3.9 shows the bar diagram for efficiency of ASP and OPG process in treating sewage.

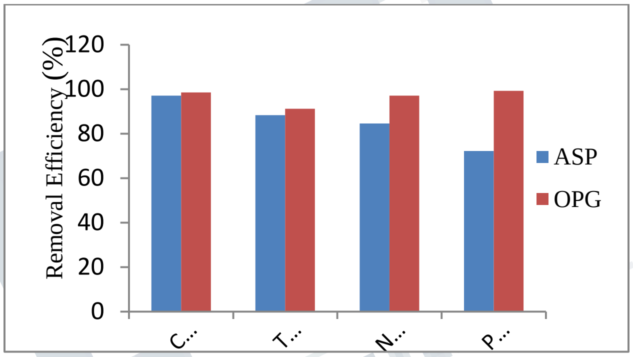

Fig 3.9 Efficiency of ASP & OPG Process in treating sewage

IV. CONCLUSION

Microorganisms in anaerobic wastewater treatment processes shown their ability to achieve a higher organic removal rate, low sludge production and lower energy consumption along with valuable methane gas production. The pilot scales study showed that the oxygenic photogranule technology is very promising compared to activated sludge process due to its advantages such as excellent settleability and small footprint. Oxygenic photogranules technology includes in-situ oxygen generation through photosynthesis with oxygen consuming processes like organic matter conversion and nitrification. Use of oxygenic photogranules for municipal wastewater treatment can make the process aeration free and energy recovery method. Oxygenic photogranules allow the recovery of chemical energy from wastewater in the form of bio-energy feedstock. Application of oxygenic photogranules for municipal wastewater treatment reduce the waste sludge generation and energy cost. The development and application of the OPG process will play a significant role in aeration-free wastewater treatment, a solar biotechnology that brings us one step closer towards sustainable treatment practices.

Dense, spherical OPGs used to treat wastewater in the laboratory have been shown to effectively remove organic nutrients such as nitrate, phosphate including COD. Therefore, the use of OPGs may significantly advance the implementation of microalgae-based wastewater treatment. Granular wastewater treatment processes have become possible in recent years through engineering the morphology of microbial communities. These granular processes offer the potential to minimize energy requirements by offering multiple conditions within each granule and thus eliminating the need for multiple treatment phases. Because OPGs harness the energy of the sun to power the transformation of carbon dioxide into much-needed oxygen, energy requirements are further minimalized.

This study is focused on a new wastewater treatment method based on the discovery of activated sludge is converted in to photogranules in the presence of source of light under static condition. This study proves that OPG can be used for high rate wastewater treatment without external aeration substantially reduces the cost of wastewater treatment. The process generates large amount of energy feed stock in the form of granules which are easily harvestable biomass. Finally this study conclude that OPG can be used as a better alternative method for activated sludge process in treating wastewater without external aeration in a cost effective manner.

V.RECOMMENDATIONS

Based on the results obtained, the following recommendations were provided:

- It is recommended that more research should be conducted on wastewater having high COD by using Oxygenic Photogranules.
- It is recommended that more studies should be conducted for investigating potential efficiency of OPGs for sewage treatment process.
- It is recommended that more research should be conducted to identify the capability of OPGs for treating various wastewaters in a cost effective manner.
- It is recommended that more studies should be conducted to prove OPGs treatment as a better alternative for activated sludge process for treating any type of wastewater.

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