Moving bed biofilm reactor technology as batch system in wastewater treatment
تكنولوجيا الطبقة الاحيائية اللزجة المتحركة كنظام مستقر في معالجة مياه الصرف الصحي

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Abstract

Biofilm slime layer is one of the advanced biological treatment technologies for industrial and municipal wastewater treatment with the capacity to reuse of treated water for agricultural purposes. Bacterial, fungal and algal biofilm slime layer were grown on the interior surfaces of polyethylene pellet (carrier) and suspended in municipal wastewater for organic pollutants removal. Bacterial species (*Pseudomonas aeruginosa, Bacillus megaterium, Sphingobacterium thalpophilum*), fungal species (*Penicillium citrinum, Aspergillus niger, Trichoderma harzianum*) and algal species (*Nostoc linckia, Scendesmus dimorphus*) were used separately for biofilm slime layer growth under controlled laboratory conditions (pH, temperature, and aeration). Bacterial biofilm layer thickness was measured and recorded 9, 6 and 5 mm respectively as compared with 3mm for control group through the retention time of 16 day. Bacterial *P. aeruginosa* biofilm slime layer showed an efficiency for COD, TOC, NO_3 and PO_4 removal after 24 hour of 75%, 65%, 69% and56% respectively while the removal rates of the same factors using the fungal biofilm layer of *P. citrinum* was 83%, 78%, 53% and 60% after 48 hour respectively. The algal biofilm reactor with *S. dimorphus* showed the highest percentage removal rate of total nitrogen 93% as compared to control group 87% after 72 hours of treatment due to the biofilm slime thickness of *S. dimorphus* 7.5mm as compared to the thickness of the *N. linckia* slime layer 5.3mm. Mixture of microbial species biofilm layer was used for wastewater treatment through 18 and 24 hours, using aerobic and anoxia. The mixture of microbial species biofilm layer showed removal rates for TOC, COD, and TN of 90%, 83%, and 59% respectively in an aerobic condition, while the removal rates were 66%, 52%, and 84% in an anoxic condition. From the above results, one concludes that controlling the biofilm slim layer is a promising technology for municipal wastewater treatment, as long as it is used under the suitable conditions.

Key words: Biofilm, wastewater

المستخلص

تعد تكنولوجيا الطبقة الاحيائية اللزجة من المعالجات البيولوجية المتقدمة لمعالجة المياه العادمة الصناعية ومياه الصرف الصحي مع امكانية لإعادة استخدام المياه المعالجة للأغراض الزراعية . تنمى الطبقة الاحيائية البكتيرية والفطرية والطحلبية على السطوح لحشوات بلاستيكية مصنوعة من مادة البولي اثيلين (سطح للالتصاق) وعلقت في احواض زجاجية مختبرية لإزالة الملوثات العضوية من مياه الصرف الصحي . استخدمت الأنواع البكتيرية (*Pseudomonas aeruginosa, Bacillus megaterium, Sphingobacterium thalpophilum*)، والأنواع الفطرية (*Penicillium citrinum, Aspergillus niger, Trichoderma harzianum*) وألانواع الطحلبية (*Nostoc linckia, Scendesmus dimorphus*) كل على حده كنموذج للطبقة الاحيائية اللزجة، ونميت في ظروف مختبرية مسيطر عليها (الدالة الحامضيةودرجة الحرارة والتهوية) .اثبتت جميع الانواع البكتيرية المستخدمة كطبقة احيائية كفاءة ازالة COD و TOC و NO3 و PO4 بعد 24 ساعة قدرتها على المعالجة حيث اظهر النوع *P. aeruginosa* افضل ازالة للعوامل البيئية اعلاه وبنسب 75٪ و 65٪ و 69٪ و56٪على التوالي. بينما كانت معدلات الازالة لنفس العوامل وباستخدام الطبقة الاحيائية الفطرية للنوع *P. citrinum* 83% و 78% و 53% و 60% على التوالي بعد 48 ساعة من المعالجة. وأظهرمفاعل الطبقة الاحيائية الطحلبية باستخدام النوع *S. dimorphus* أعلى كفاءة إزالة لقيمة النيتروجين الكلي وبنسبة 93% مقارنة بمجموعة السيطرة 86% بعد 72 ساعة من المعاملة . هذا بسبب سمك الطبقة الاحيائية الطحلبية للنوع *S. dimorphus* (7.5mm) مقارنة مع سمك الطبقة سمك الطبقة الاحيائية الطحلبية للنوع *N. linckia* (5.3mm). استخدم خليط من الأنواع الاحيائية المنماة كطبقة احيائية هلامية لمعالجة مياه الصرف الصحي بعد 18 و 24 ساعة وفي ظروف هوائية وقلة الأكسجين حيث سببت هذه الطبقة الاحيائية نسب إزالة 90% و 83% و 59% للعوامل TOC و COD و TNفي الظروف الهوائية على التوالي بينما كانت نسب الإزالة 66% و 52% و 84% في ظروف نقص الأوكسجين . يتبين من النتائج اعلاه قدرة تكنولوجيا الطبقة البيولوجية الواعدة على معالجة مياه الصرف الصحي مادامت تعمل في الظروف المناسبة .

الكلمات المفتاحية: الاحيائية اللزجة ، مياه الصرف الصحي

Introduction:

Biofilm layer is defined as the dense aggregates of surface-adherent microorganisms (mostly bacteria) which embedded in an extra polysaccharide matrix or substance (EPS) [1]. The EPS, in turn, is normally composed of 40-95% polysaccharides, 1-60% proteins, 1-10% nucleic acids and 1-40% lipids [2]. The composition of the EPS varies with the composition of the microbial consortia and the environmental conditions [3].

Biofilm development is a result of successful attachments and subsequent growth of microorganisms on the internal surface of polyethylene pellet. Biofilm formation is a multi step process whereby microorganisms, adhere on the surfaces, surround themselves with a protective layer of polysaccharides and grow into a network of micro colonies with water channels. The rate of biofilm formation can be affected by many factors such as surface characteristics, availability of nutrients and flow velocities. A biofilm continues to grow until the surface layers begins to slough off into the water [4].

The moving bed bacterial and fungal biofilm reactor (MBBR) is a biofilm treatment system which is capable of degrading organic compounds and nutrients (N and P) in wastewater effluents by employing various metabolic and respiratory processes [5]. The key feature of the MBBR function is by using a small polyethylene plastic biofilm support media to allow for a high concentration of biofilm to growth in a well mixed reactor vessel [6].

Municipal wastewater is composed of organic material (proteins, carbohydrates, fats and oils); nutrients, mainly nitrogen and phosphorus; as well as trace amounts of recalcitrant organic compounds and metals [7]. Biodegradable organic material is biochemically oxidized by heterotrophic bacteria under aerobic conditions leading to the production of carbon dioxide, water, ammonia and new biomass. Under anaerobic conditions methanogenic archaea, partially oxidizes organic material to yield carbon dioxide, methane and new biomass [8].

The objective of this study is to evaluate the environmental factors removal rates of (COD, BOD_5, NO_3 and PO_4) by applying a lab-scale MBBR system filled with biofilm carriers (pellets),the biofilm consist of different isolates of microorganism (bacteria, fungi and algae) with the changing of environment conditions in wastewater treatment.

Materials and Methods

-Isolation and identification of microorganisms

Three main types of microorganisms were used to form the biofilm slime layer used in the treatments of pollutants in this study.

1. Bacteria: Three bacterial species *Pseudomonas aeruginosa* , *Sphingobacterium thalpophilum*, *Bacillus megaterium* were isolated from sewage water in addition to other sources of water. These bacterial species were identified by using the Vitek Compact 2 equipment.

2. Fungi: The fungal species *Penicillium citrinum*, *Trichoderma harzianum*, *Aspergillus niger* were isolated from sewage water, then were cultured on the various media (PDA and Corn meal agar). These fungi identified from standard morphological characteristics [9,10]

3. Algae: Algal species *Scendesmus dimorphus* and *Nostoc linckia* were isolated from sewage water samples, cultivated on modified chu-10 medium [11] at controlled environmental conditions in photo incubator (16:8 hour light: dark regime; light 245 µEm-2s-^{1}; temp. 25 ± 2c$^{\circ}$). The Algal species were diagnosed by compound microscope in addition to the scientific references [12].

-Chemical analysis: The chemical analysis that were used to determine the pollutant concentrations before and after the treatments of the wastewaters are soluble COD, BOD5, TOC, nitrate (NO3-N), and phosphorus (PO4-P), these factors were measured in accordance with standard methods for the examination of water and wastewater (American public health association [13], While Total phosphate and total nitrate were measured according to [14].

- Culturing of biofilm on Biocarrier (pellets): Microorganisms (bacteria, fungi and algae) were grown aseptically on biofilm carrier elements made from high density polyethylene and have a specific gravity of 0.96 (MBBR)(Figure 1a) in a large glass tanks for period of 10-16 days in batch system, where the tank was aerated from above of tank by a stone diffuser connected to a pressure and flow controlled air stream(Figure 1b). Then the growing biomass on bio-carrier was used for wastewater treatment.

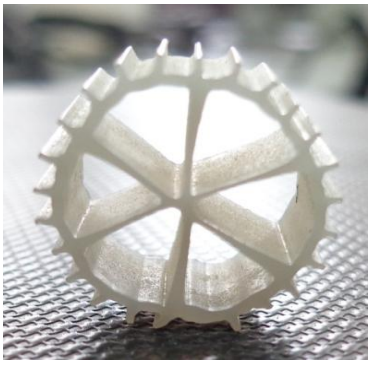
A  B 

Fig. (1): Moving bed bioreactor system. (A) biocarrier, (B) Tank of the reactor.

-Bacterial biofilm: Samples were taken from station of (Saba Abkar) for wastewater treatment, these waste water were placed in volumetric glass bottles size 1L at quantity of 500ml, then sterilized by autoclave device for 20 min to killing of microorganisms. After cooling the samples, the pH was adjusted to 6.5 by using concentrated sulfuric acid, suitable aeration, with the addition of nutrients K_2HPO_4 0.1g/L as phosphate source, NH_4NO_3 (1g/L) as nitrogen source, glucose (1g/L) as carbon source in addition to bacterial biomass were measured in spectrophotometer equipment at 3ml for each bottle 60 pellet/L. The environmental factors NO_3, PO_4, BOD_5, and TOC were measured before and after 24, 48 hour from treatment.

-Fungal biofilm: Samples were taken from wastewater treatment station, these samples were placed in volumetric glass flasks (1L), sterilized by autoclave equipment for 20 min to killing of microorganisms, cooling and was adjusted to the pH of 5.8, and addition of the suitable nutrients such as K_2HPO_4 (0.1 g/L) as phosphate source, NH_4NO_3 (1g/L) as nitrogenous source and glucose (1g/L) as carbon source. Fungal biomass was added at 5g/500ml sample for each fungal species grown on polyethylene pellet (50 pellet /L). Three replicate were done for each species. After 48 and 96 hour from wastewater treatment, the environmental factors (NO_3, PO_4, BOD_5 and TOC) were also measured in addition to their removal rate.

-Algal biofilm: Samples were brought from station of (Saba Abkar) in the city of Baghdad for wastewater treatment, those samples were placed in a volumetric glass flasks (250 ml), sterilized by autoclave equipment for 20 min to kill microorganisms and left for cooling. A 0.08 g / l of algal biomass of *S. dimorphus* and *N. linckia* growing on pellets at average of 43 algal thread / mL measured by haemocytometer was added to the sample above. For each flask 15 biofilm pellets was added at an average of 3 replicates. The average weights of empty pellet were recorded as 1.02 grams. The environmental factors and removal rates were also measured for sewage water after 24, 48 and 72 hours of the treatment process.

Result and Discussion

In the last decades the MBBR was invented to be an effective alternative wastewater treatment process that could provide advantages over activated sludge or other biofilm technologies. The principal of the moving bed biofilm reactor (MBBR) treatment process is the treatment of the incoming wastewater by microorganisms growing on bio-carriers freely suspended in the mixed liquor of the MBBR reactor, bio-carrier movement within the reactor is produced by an engineered aeration system.

Bacterial biofilm layer

MBBR technique utilized for the treatment of organic pollutions of wastewater through the formation of bacterial biofilm slime layer on the pellet. Table (1) shows the reduction rates of environmental factors after 24 hours of treatment by bacterial biofilm for isolates *P. aeroginosa, S. thalpophilum and B. megaterium* separately as compared with control (sewage water). Different nutrients were added to each bioreactor under suitable aeration, this is done through the aerator which provides O_2 and good mixing [15]. The results showed that the highest removal of COD, TOC, nitrate and phosphate in wastewater was achieved by using biofilm *P. aeroginosa* with the rates of 65%, 75%, 69% and 56% when comparing with the control group after 24 hour from treatment respectively. Meanwhile biofilm *B. megaterium* and *S. thalpophilum* came in the second and third state in the removal of the environmental factors respectively. This is due to the ability of these bacteria for biodegradation of different pollutants and their

rapid growth and reproduction on plastic pellet under appropriate conditions [16]. There were many factors that contributed in the increment of the pollutants removal rates and efficiency such as thickness of attached biofilm layer, availability of oxygen and nutrients, mixing process and retention time [17]. Biofilm thickness was found to be an essential factor in this treatment, from the results above the biofilm *P. aeroginosa* was recorded the highest removal rates of pollutants due to their biofilm thickness 9mm as compared with *B. megaterium*, *S. thalpophilum* and control were recorded 6 mm, 4 mm and 3mm during the 16-18 days of development respectively. The *P. aeroginosa* is gram negative bacteria, its ability for attaching on the biotic and abiotic surfaces and formation of biofilm layer is due to its content of protein system [18]. The availability of nutrients in the wastewater influent contributed in the abundance growth, reproduction, composition of the bacterial biofilm and the production of extracellular polysaccharide (EPS) [19]. The other factor that contributed in the increasing of the pollutants removal rates and efficiency was mixing process of the biofilm reactor which provided both sufficient oxygen and uniformity of the waste mixture which had its positive effects on the growth rates of the biofilm formation [20]. Also the retention time was considered one of the essential factors in the treatment due to their allowing for the bacteria to grow and reproduction [21].

Table (1): Removal rate for environmental factors of sewage water by using different isolates of bacterial biofilm after 24 hour from treatment.

Type of bacterial biofilm	Environmental factors				Biofilm Thickness mm
	PO_4%	NO_3%	TOC%	COD %	
Control	**38**	**53**	**33**	**50**	**3**
B. megaterium	**51**	**41**	**36**	**62**	**6**
S. thalpophilum	**29**	**37**	**31**	**52**	**4**
P. aeroginosa	**56**	**69**	**65**	**75**	**9**

Table (2) shows the removal rate of TOC, COD and nutrients in wastewater by bacterial biofilm after 48 hours of treatment, which biofilm *P. aeroginosa* was recorded a remarkable increase in the removal rate of COD, TOC, nitrate and phosphate as follows 78%, 84%, 73% and 87% due to the high density and thickness of biofilm *P. aeroginosa* 9.5 mm comparing with 6.3mm, 4.1mm and 3.1 for *B. megaterium*, *S. thalpophilum* and control after 48 hr. of treatment respectively. Biofilm thikness of bacterial species was considered positive factor in the treatment of organic pollutants in wastewater, which the microbial thickness is depended on the microbial species, type and source of sewage water [22].The study appeared that adding of long-term nitrate in fact stimulate the effectiveness of bacteria reducing nitrate in biofilm of sewage water [23], also nitrate consumption was increased by bacterial biofilm with re-exposure to nitrate in the samples and conversely increasing of COD consumption in wastewater. The genus *Sphingobacterium* is widely distributed in the environment this attributed to their ability to metabolize a large variety of organic compounds in addition to its surviving and growth under oligotrophic or starvation conditions [24]. The genus *Sphingobacterium* is identified as important members of biofilm communities in freshwater habitats, their ability to swimming and motility due to spore forming and facilitate colonization on the surface of RO membranes. This isolate grew in much quantity on RO membranes as biofilm layer, presumably due to their ability to produce EPS [25]. Lab-scale moving bed bacterial biofilm reactors are shown in Figure (2).

Table (2): Removal rate for environmental factors of sewage water by using different isolates of bacterial biofilm after 48 hour from treatment.

Type of bacterial biofilm	Environmental factors				Biofilm Thickness (mm)
	PO_4(%)	NO_3 (%)	TOC (%)	COD (%)	
Control	**67**	**61**	**58**	**66**	**3.1**
B. megaterium	**56**	**69**	**56**	**75**	**6.3**
S. thalpophilum	**42**	**53**	**51**	**62**	**4.1**
P. aeroginosa	**78**	**84**	**73**	**87**	**9.5**

Fig (2): Bacterial biofilm reactor for isolates *B. megaterium, S. thalpophilum* and *P. aeroginosa.*

Fungal biofilm layer

Table (3) shows the removal rate of organic pollutants by reducing the concentrations of COD and TOC in sewage water after 48 hour from treatment with fungal biofilm reactor such as *T. harzianum, A.niger* and *P. citrinum* without aeration. These results demonstrated that the fungal *P. citrinum* biofilm reactor caused the highest removal rates of COD, BOD_5, TOC, NO_3 and PO_4 at rate of 83%, 89%, 78.18%, 53% and 60% after 48 hour from wastewater treatment respectively, but the other two types of fungal reactor recorded varying rates of removal for environmental factors. These due to the efficiency of *P. citrinum* biofilm on the sewage treatment with the use of organic pollutants as sources of energy for growth in addition to the wide surface area of the fungal biofilm on the inner surfaces of pellet (biocarrier) and forming suitable biofilm thickness 8.1mm as compared with other species and control 7.5,7.4,4mm respectively. The amount of pellet surface filling with biofilm influenced by the concentrations of organic carbon in sewage water, when increasing the concentrations of organic carbon (increasing COD), these lead to the increasing the growth and reproduction of microorganisms constituent biofilm layer on the internal surface of pellet and vice versa [26].

Both fungal species of *P. expansum* and *P. brevicompactum* contributed in formation of biofilm slime layer in drinking water distribution pipes, these fungi are most important environmentally due to their overlap and interaction with the other microorganisms present in abundance in water [27]. Table (4) shows the removal rates of organic matter and nutrients in wastewater by biofilm fungal reactor without aeration after 72 hr. of treatment. These results proved the efficiency of fungal biofilm reactor in the removal rate of the environmental factors as compared to control group, while [22] recorded the BOD_5 removal rate at percent of 78% in MBBR reactor as compared with 94% removal rate for this result. Attachment of microorganisms on plastic surfaces and formation of fungal biofilm layer take 48 hour under incubation temperature of 37 C° and differs according to the microorganisms used in the treatment [28]. Lab-scale moving bed fungal biofilm reactors are shown in Figure (3).

Table (3): Removal rate for environmental factors of sewage water by using different isolates of fungal biofilm after 48 hour from treatment.

Type of fungal biofilm	Environmental factors					Biofilm Thickness(mm)
	PO_4 %	NO_3 %	TOC%	BOD_5 %	COD%	
Control	56	41	36	67	72	4.0
P. citrinum	60	53	78	89	83	8.1
T. harzianum	58	50	78	88	77	7.5
A. niger	49	25	64	81	74	7.4

Table (4): Removal rate for environmental factors of sewage water by using different isolates of fungal biofilm after 72 hour from treatment.

Type of fungal	Environmental factors	Biofilm

biofilm	PO_4%	NO_3 %	TOC%	BOD_5 %	COD%	Thickness(mm)
Control	62	50	55	69	80	4.2
P. citrinum	89	75	93	94	93	8.2
T. harzianum	73	69	88	93	86	7.7
A. niger	56	33	82	92	91	7.7

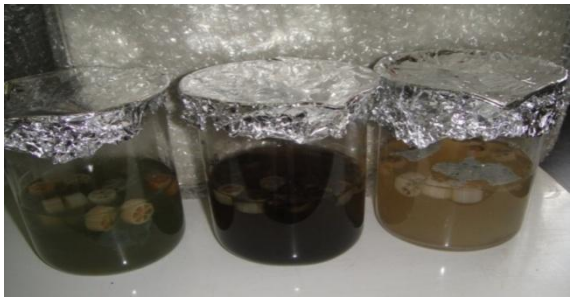


Fig (3): Fungal biofilm reactor for isolate *T. harzianum, A. niger* and *p. citrinum*.

Algal biofilm layer

Figures (4a,b) Illustrates the total nitrogen removal rate from sewage water by *S. dimorphus* and *N. linckia* algal biofilm after 24, 48, 72 from treatment as comparing with the control group respectively. The results showed the efficiency of algal biofilm reactor in the removal of total nitrogen with the highest removal rate of 93.39% and 64.89% for *S. dimorphus* and *N. linckia* after 72 hours of treatment as compared to control group 86.56% respectively, this due to the thickness of *S. dimorphus* biofilm (7.5mm) while the thickness of *N. linckia* biofilm 5.3mm. In Figure (5a,b) the highest removal rate of total phosphorus in wastewater was by using *S.dimorphus* biofilm (53.1%) as compared with *N. linckia* biofilm 36.86% after 72 hours of treatment. Meanwhile, the results showed an increase in weight of *N. linckia* biofilm pellet (4.125 g) after seven days of treatment while weight of *S. dimorphus* biofilm pellet was recorded 1.67 g. These results suggested that the developing algal density (large surface area) on plastic fillings due to the consumption of nutrients in sewage and their efficiency in reducing their rates without side effects. Several studies indicated the role and importance of algae in the removal of inorganic contaminants (phosphorus and nitrogen) from sewage water [29]. The removal of nutrients from wastewater are completely done by using algae and never let any area of contamination in the treatment process and in the recent years, many of researchers concentrate their researches on the using of algae as an alternative method for secondary treatment in sewage treatment and is considered as one of the methods of environmentally friendly but the deposition of algae after water treatment is a problem in its removal [30]. Also, many researches are directing on the using of immobilized and attaching algal cells on the substrate (pellet) to solve this problem with the sedimentation of algal cells is unnecessary and the metabolic events remain stable for long periods, with using of the harvested algal biomass as a food source or chemical material [31].

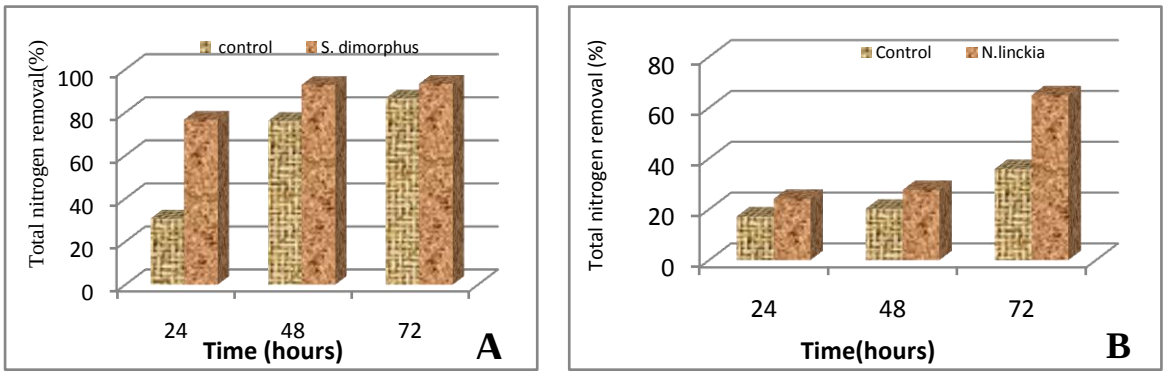


Fig (4): Removal rate (%) of total nitrogen by algal biofilm *S. dimorphus* (A) and *N. linckia*(B) through different time periods of treatment.

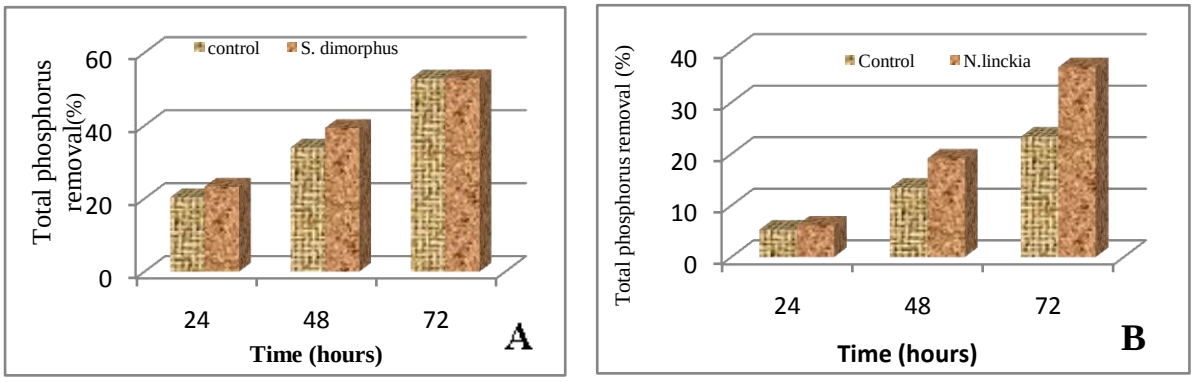


Fig (5): Removal rate (%) of total phosphorus by algal biofilm *S. dimorphus* (A) and *N. linckia* (B) through different time periods of treatment.

The results in Table (4) display the removal rates of environmental factors in wastewater by using biofilm reactor growing on the internal surfaces of moving pellets (as carrier) in aerobic conditions and with addition of external carbon source (pepton and methanol) after 18 and 24 hours of wastewater treatment. The removal rates of TOC and COD were 90% and 83% after 24 hr. from treatment respectively, due to efficiency of bacterial and fungal biofilm reactor on the consumption of organic carbon for the energy and growth while algal biofilm contribute in the consumption of phosphates and nitrogen in sewage as basic nutrients and reducing their rates to 85% and 59% respectively under favorite pH for microorganisms growth in the aquatic environment. The biomass that sloughs off from biocarrier then passes through the effluent sieve of treatment tanks after that the clarification/sedimentation is then employed to remove the sloughed off biomass from the treated wastewater. Figures (6 a, b) shows the moving bed biofilm reactor in glass cylinders containing of the wastewater. Addition of external nutrients such as glucose, methanol or acetate are considered an essential for growth and reproduction of microbial biofilm with enhanced the process of denitrification (removal of nitrate) [32]. Tam *et al*. [33] was used methanol as industrial standard material in the denitrification from wastewater because of little cost and gives appropriate reactions and produces few cells.

Table (4): Moving bed biofilm reactor in aerobic condition for wastewater treatment.

No.	Environmental factors	Before treatment ppm	After treatment ppm		Removal rate % after 24hr.
			18hr.	24hr.	
1	TOC	25	12.4	2.6	90
2	COD	950	554	160	83
3	TN	15	10	6.2	59
4	TP	5.4	3.10	0.8	85
5	DO	0.08	2.4	4.2	-
6	pH	7.2	8.2	9.1	-

The results in Table (5) showed that the thickness of biofilm layer was recorded the removal rate for TOC and total nitrogen of % 94 and 84% through anoxic conditions and after 18,24 hours of treatment respectively. The significant reduction of nitrate through this analogical period with the lack of oxygen was attributed to the activity and efficiency of microbial biofilm in the reduction of nitrate by denitrification process and converts all forms of nitrogen into nitrogen gas with the using of external organic carbon for nitrate reduction. The concentrations of COD were increased during the periods of the treatment due to the external carbon source; in addition the biological treatment was decreased because of oxygen depletion that essential for oxidation of organic matter. Also, the results displayed a dispersed in phosphate concentrations during the treatment periods these may be due to the lack of oxygen or because of the acidic condition. Figure (6) showed the biofilm reactor in aerobic and anoxic condition with using of different nutrient sources such as methanol and peptone. The glucose and the combination of soya peptone with yeast extract at a temperature of 45 c° are considered optimal conditions for cell division and formation of slime biofilm with the production of exopolysaccharide materials [34]. The oxygen produced by algae metabolism is essential and necessary in the oxidation of organic matter by bacteria and fungi in the biofilm [35]. The peptides and amino acids are considered the essential elements favorite and encouraging the development of large amounts of biofilm [36]. Figure (7)

displayed the thickness of microbial slime biofilm for community of microorganisms (bacteria, fungi and algae) grown on pellet after 10 days from treatment.

Table (5): Moving bed biofilm reactor in anoxic condition for wastewater treatment.

No.	Environmental factors	Before treatment ppm	After treatment ppm 18hr.	24hr.	Removal rate %
1	TOC	25	8.2	1.6	94
2	COD	950	754	460	52
3	TN	15	8	2.4	84
4	TP	5.4	9.2	12.5	-
5	DO	0.08	0.05	0.04	-
6	pH	7.2	7.5	7.8	-

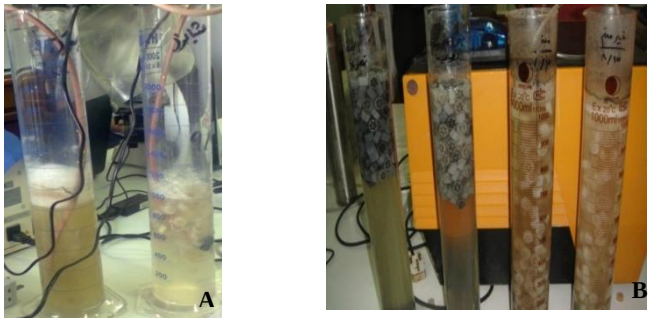


Fig (6): Moving bed biofilm reactor in aerobic and anoxic condition (A), different nutrient sources (methanol and pepton) (B).

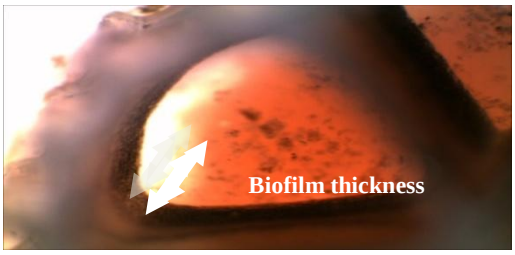


Fig (7): Thickness of biofilm slime layer for community of microorganisms (bacteria, fungi and algae) on pellet after 10 days from treatment.

Conclusion

The following conclusions can be drawn from the data obtained from the experiments; the efficiency of this technology is an important factor and a key to the recovery and recycling of contaminated water; MBBR technology is low-cost technologies that lead to major tasks in biological treatments for wastewater; The possibility of replacing traditional techniques such techniques for newly built projects in villages and remote areas within the evolution of this process.

References

1. Stoodley, P., Sauer, K., Davies, D.G. and Costerton, J.W. (2002). Biofilms as complex differentiated communities. Annu Rev Microbiol. 56:187-209.
2. Flemming, H-C and Wingender, J. (2002). Extracellular Polymeric Substances (EPS): Structural, Ecological and Technical aspects, in Encyclopedia of environmental microbiology, Bitton G, Editor. John Wiley & Sons: New York. p.1223-1231.
3. Liu, H. and Fang, H.H.P. (2002). Extraction of extracellular polymeric substances (EPS) of sludges. Journal of Biotechnology. 95, 249-256.
4. Geldreich, E.E. and Rice, E.W. (1987). Occurrence, significance and detection of Klebsiella in water systems.J. American Water Works. Assoc. 79: 74.
5. **5.** Cucarella, C., Solano, C., Valle, J., Amorena, B., Lasa, I. and Penadés, J. R. (2001). Bap, a *Staphylococcus aureus* Surface Protein Involved in Biofilm Formation.J Bacteriol. 2001 May. 183(9): 2888–2896.

6. Ødegaard, H., Gisvold, B., and Strickland, J. (2000). The influence of carrier size and shape in the moving bed biofilm process. Water Sci.& Technol. 41 (4-5) 383-391.

7. Zimmo, O.R., Steen, V.D. and Gijzen, H.J. (2004). Nitrogen mass balance across pilot-scale algae and duckweed-based wastewater stabilization ponds. Water Res. 38(4): 913-920.

8. Cao, B., Shi, L., Brown, R. N., Xiong, Y., Fredrickson, J. K., Romine, M. F., Marshall, M. J., Lipton, M. S. and Beyenal, H. (2011). Extracellular polymeric substances from *Shewanella* sp. HRCR-1 biofilms: characterization by infrared spectroscopy and proteomics. Environmental Microbiology. 13: 1018–1031.

9. Raper, K.B. and Fennell, D.I. (1965). "The genus Aspergillus". Williams & Wilking Co., Battimore. 686-754.

10. Barnett, H.L. and Hunter, B.B. (1972)."Illustrated genera of imperfect fungi". 3rd ed. Burgess publ. co., Minnesota. (1972).

11. Kassim, T.I., AL-Saadi, H.A. and Salman, N.A. (1999). Production of some phyto and zoo plankton and their use live food for fish larva. Inpress.1-21Pp.

12. Patterson, G. (1983). Effect of heavy metals on fresh water Chlorophyta. Ph.D. thesis. Univ. Durham. 212Pp.

13. APHA. (2005). Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association, Washington, DC.

14. Standard Methods For The Examination of Water and Wastewater, Method 4500-NO3, Nitrate Nitrogen Automated Cadmium Reduction Method, 4500-P Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus. American Public Health Association. 21st Edition, 2005.

15. Z mat, A.D. and Ghawi, A.H. (2009). Performance prediction of rotating biological contractor in wastewater treatment applications. Al-Qadisiya J. for Eng. Sci. 2 (2):164-176

16. Leiknes, T. and Ødegaard, H. (2007). The development of a biofilm membrane bioreactor. Desalination. 202: 135-143.

17. Kermani, M., Bina, B., Movahedian, H. Amin, M. M. and Nikaeen, M. (2009). Biological phosphorus and nitrogen removal from wastewater using moving bed biofilm process. Iranian J. of Biotechnol. 7(1): 256- 262.

18. Mace, C, Seyer, D., Chemani, C ., Cosette, P., Di-Martino, P., Guery, B., Filloux, A., Fontaine, M., Molle, V. and Junter, G-A. (2008). Identification of biofilm-associated cluster(bac) in *Pseudomonasaeroginosa*involved in biofilm formation and virulence. PLoS ONE. 3(12):e3897.

19. Rochex, A. and Lebeault, J.M. (2007). Effects of nutrients on biofilms formation and detachment of Pseudomonas putida strain isolated from a paper machine. Water Res. 41: 2885-2892.

20. Berman, T. and Passow, U. (2007). Transparent Exopolymer Particles (TEP): an overlooked factor in the process of biofilm formation in aquatic environments. Nature Precedings : doi:10.1038/npre.2007.1182.1.

21. Calderón, K., Martín-Pascual, J., Poyatos, J.M., Rodelas, B., González-Martínez, A., González-López, J. (2012). Comparative analysis of the bacterial diversity in a lab-scale moving bed biofilm reactor (MBBR) applied to treat urban wastewater under different operational conditions. Bioresour Technol. 121:119-26.

22. Abdul –Majeed, M.A., Alwan, H.H., Baki, M.I ., Abtan, F.R and Sultan, H.I. (2012). Waste water treatment in Baghdad city using moving bed biofilm reactor (MBBR) technology. Eng. &Tech. Journal. 30(9): 1550-1561.

23. Mohanakrishnan, J., Gutierrez, O., Sharma, K.R., Guisasola, A., Werner, U., Meyer, R.L., Keller, J. and Yuan, Z. (2009). Impact of nitrate addition on biofilm properties and activities in rising main sewers. Water Research. 43 17: 4225-4237.

24. Koskinen, R., Ali-Vehmas, T., Kampfer, P., Laurikkala, M., Tsitko, I., Kostyal, E., Atroshi, F., Salkinoja-Salonen, M. (2000). Characterization of Sphingomonas isolates from Finnish and Swedish drinking water distribution systems. J. Appl. Microbiol. 89, 687-696.

25. Balkwill, D. L., Fredrickson, J. K., Rominem, M. F. (2000). *Sphingomonas* and related genera. In The Prokaryotes: an evolving electronic resource for the microbiological community; Dworkin, M., et al.,Eds.; Springer-Verlag: New York. http://link.springerny.

26. Jun, H.B., Kim, B. K., Seo, I. S. and Lee, S. H. (2000). COD andnitrogen removal from sewage in a modified intermittent aeration process MIAP. J. KSWQ. 57–64.

27. Goncalves, A.B., Paterson, R.R.M., Lima, N. (2006). Survey and significance of filamentous fungi from tap water. Int J Hyg Environ Health. 209, 257-264.

28. Martinez, L.R., Mihu, M.R., Han, G., Frases, S., Cordero, R.J.B., Casadevall, A., Friedman, A.J., Friedman, J.M. and Nosanchuk, J.D. (2010).The use of chitosan to damage Cryptococcus neoformans biofilms.Biomaterials. 31(4): 669-679.

29. Aslan, S. and Kapdan, I.K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecological Engineering. 28(1): 64-70.

30. Beuckels, A., Smolders, E. and Muylaert, K. (2012). Wastewater treatment using microalgae: optimizing removal of P from wastewaters with a low total N:P ratio. J. Algal Biomass Utln. 3 (2): 9- 13.

31. Lukavaski, J. (1986). Metabolic activity and cell structure of immobilized algaecells. Algalgical studies. 43:261-279.

32. Peng, Y.Z., Ma, Y. and Wang, S.Y. (2007). Denitrification potential enhancement by addition of external carbon sources in a pre-denitrification process. J. Environ.Sci.-China.19(3): 284-289.

33. Tam, N.F.Y., Leung, G.L.W. and Wong, Y.S. (1994). The effect of external carbon loading on nitrogen removal in sequencing batch reactors. Water Science and Technology. 30(6): 73-81.

34. Berenjian, A., Chan, N.L., Mahanama, R., Talbot, A., Regtop, H., Kavanagh, J. and Dehghani, F. (2012). Effect of Biofilm Formation by *Bacillus subtilis* natto on Menaquinone-7 Biosynthesis.Mol Biotechnol. 28:146-158.

35. Deng, X., Wei, B. and Hu, Z.L. (2011). Simultaneously removal of COD, nitrogen and phosphorus from wastewater by coupling treatment system with immobilized algae-bacteria. Huan Jing Ke Xue. 32(8):2311-6.

36. Ramli, N.S.K., Guan, E. C., Nathan, S., Vadivelu, J. (2012). The Effect of Environmental Conditions on Biofilm Formation of *Burkholderia pseudomallei* Clinical Isolates. PLoS ONE 7(9):e44104. doi:10.1371/journal.pone.0044104.