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Study of the efficiency of cyclic activated sludge process with moving bed in treatment of the municipal wastewater. (Case study: Removing the antibiotic amoxicillin from wastewater)

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ABSTRACT

Introduction: Pharmaceutical materials and antibiotics are a part of emerging pollutants in aqueous environments, which threaten human and environmental health at low environmental concentrations. Amoxicillin is a most-used antibiotic in the treatment of infections most of which is disposed as non- metabolized, then enters into wastewater and finally come into the environment.

Materials & Methods: Present study was conducted with the aim of investigating the process of cyclic activated sludge with moving bed in removing amoxicillin from aqueous environments. Following preparing amoxicillin and other chemical compounds in this experimental study, first required reactor at pilot scale with volume of about 100 L was designed, prepared, equipped and sealed. Then prepared system was transferred to urban wastewater treatment plant and installed there.

Results: Regarding low value of antibiotic in urban wastewater, after initial setting up, fixing possible defects and stability of reactor, amoxicillin was fed into the inlet wastewater by adding concentrations of 5, 10, 15 and 20 mg/L and then sampling, preparation, samples filtration and making required trials were done and subsequently concentration of amoxicillin was obtained through HPLC system. In order to analyze statistical data in Excel software, efficiency of the reactor of cyclic activated sludge with moving bed in removing amoxicillin from wastewater was evaluated. Based on the obtained results and the best operating conditions of the system, the highest rate of removing amoxicillin pollutant with inlet concentration of 5mg/L and 12 hours Hydraulic retention time of 94.3% were achieved.

Conclusion: Regarding suitable conditions of model in removing pharmaceutical pollutants and other organic materials, applying this method in designing and exploiting MBAS system was suggested.

Keywords: Amoxicillin, Antibiotic, Rotating Bed, Cyclic Activated Sludge

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Introduction

During recent years, due to spreading diseases, advances in medical sciences, pharmacology and therapeutic coverage, taking medicines and availability of many types of medicines have been increased in the world. Iran is one of the largest drug users in the world (1, 2). so that is regarded as one of 20 top countries in the world in terms of taking medicines and in Asia it stands in second place after China (2,9,8). According to WHO estimates in 2006, 7700 Kg antibiotic is produced in the world daily (8, 3). In terms of consumed vials, antibiotics are considered to be the most used pharmaceutical groups in Iran so that it is about 15% of total consumed medicines (2, 10). More than 65% of consumed antibiotics in the world are beta lactam group (amoxicillin, Penicillin, Ampicillin) among which Amoxicillin is most used in the world (5, 4). In Iran, 32.6% of antibiotic consumption belongs to Beta Lactam group among which Amoxicillin is the most used medicine (13, 6). Amoxicillin is a semi synthesized penicillin with a beta Lactam cycle and the molecular weight of 365.4 g/ mole which prevents synthesizing bacteria's cell wall (14, 13, 8, 7) which is used as bacteriostatic in removing infectious bacteria (6, 12). Increasing consumption of antibiotics has caused rising concern about presence of a wide variety of pharmaceutical materials in aqueous environments. Usually, due to inefficiency of common technologies in wastewater treatment, these materials have entered into the environment through outlet effluents (9, 14). Antibiotics are stable and lipophilic and due to continuous entry into the environment they can survive there for long terms whose presence is hazardous in both low and high concentrations. These compounds are so resistant to biological decomposition (11, 17). The significance of antibiotics lays in making bacterial resistance so that they are considered to be a threat for human's health (18, 19, and 12). Antibiotics prevent effective treatment of wastewater therefore wide usage of these medicines can lead to rising environmental hazards. Moreover, direct presence of these compounds in pharmacology wastewater can influence on biological treatment and microbial population of reactors. Remaining antibiotics and their metabolite in sludge can have negative effect upon treatment system like anaerobic digester and nitrification systems (19, 13). In a study by Amin et al. in 2006 it was indicated that long-term exposure to anti-microbes may cause changing in microbes' population which can have a negative impact on anaerobic treatment efficiency (20, 14). In a study by Hashemi et al. in 2010 entitled "The Impact of Antibiotics on Activity of Anaerobic Biomass Methane Generating" it was showed that amoxicillin has a preventive role in anaerobic biomass methane generating activity at concentration of 9000 mg/L (21, 15). Therefore, because of high usage of antibiotics, their concentrations have raised in the water that causes deteriorating water quality so that several studies are available confirming antibiotics concentration of 100 microgram/L within surface water and groundwater environments. On the other hand, industries for antibiotic production have produced antibiotic effluents with high concentration of 100 mg/L (28, 22). Wastewater treatment by a biological way is one of most used methods for wastewater treatment in the world. In this way, microorganisms play a significant role and if they are affected by antibiotics, the process of biological treatment will be disturbed (21, 20). These antibiotics don't tend to be absorbed by soil and by penetrating into earth's crusts enter the groundwater. Therefore, it is necessary to remove antibiotics from pharmacology, urban wastes, agricultural and industrial effluents (16, 17, and 26). Urban wastewater systems as a main pathway of antibiotics disposal and measured concentrations in the outlets of wastewater treatment units can reflect antibiotic values in the receiving region (19, 13). Investigation of internal and external sources indicate that no previous study has been done upon suggested process for removing pharmaceutical wastewaters. The difference between this system and combined reactor is that in combined reactors applied in the world until now, there is need for separate settling basin or they are handled discontinuously. In the cyclic single-basin activated sludge system offered in this study, a combined reactor has been applied with a continuous flow in a basin and without external settling. In present study, it is focused upon Amoxicillin as an antibiotic with wide variety applied in curing humans and animals whose presence in urban wastewater treatment units has been reported and its concentration of 120 ng/L in wastewater treatment plants has been reported in monitoring plans implemented in Italy and the aim of this study is to investigate the efficiency of cyclic activated sludge with moving bed in removing Amoxicillin from wastewater.

Materials and methods

Pilot characteristics (device description)

This reactor is composed from a 100L tank consisting a series of equipment and accessories such as feeding tank, a sewage inlet valve, a sewage outlet valve equipped with automated valve, air injection valve, a sludge drain valve and sewage overflow valve. In this reactor, the operation of sewage inlet is done continuously and its outlet is done discontinuously. Aeration and sedimentation in this system is done through a basin as a continuous cycle. Required air for supplying dissolved oxygen and media rotating is fed into the whole reactor volume through an air compressor and four air conditioners located at the bottom of reactor. In order to prevent media outlets, a mesh screen installed at reactor outlet is applied. Also Kaldnes media (K1) (Pakan Qatreh, Iran) made from polyethylene (PE) with special weight of 0.97 g/cm3 and media's special nominal surface of 500 m2/m3 are applied as a bed for examining the efficiency of this system in removing amoxicillin. Schematic pilot of MBAS is illustrated in figure1.

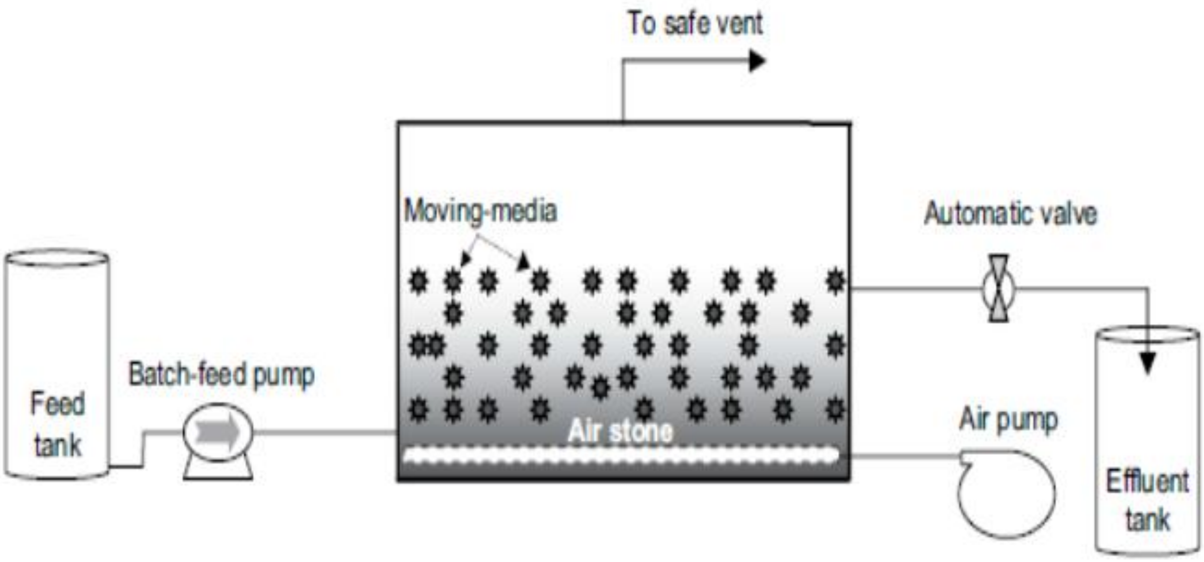

Fig 1. Schematic applied in this study

Set up and Stability of Reactor MBAS

In order to initial set up of reactor, prepared pilot system was transferred to urban wastewater treatment plant and installed at that place. First, about 20% of its volume was filled with rotating media. In order to accelerate reactor stability, more than one third of reactor volume was filled with activated sludge obtained from returned sludge from secondary settling basin of wastewater treatment plant and then system loading with natural (actual) sewage was applied. Regarding the low value of antibiotic in urban sewage, after initial set up, fixing possible defects, reactor's setting up with retention times of 4, 8 and 12 hours through different cycles of 4, 6, 8 and 10 hours and then reactor's optimal stability in hydraulic retention time of 8 hours during a 8 hours cycle (6 hour aeration, 1.5 hour sedimentation and 0.5 hour sludge drain) by adding concentrations of 5, 10, 15, and 20 mg/L of amoxicillin into inlet sewage and then sampling, preparation, samples filtration and making required trials, the concentration of amoxicillin was measured by HPLC. In order to data analysis by using software EXCEL and SPSS, efficiency of cyclic activated sludge reactor with rotating bed was evaluated in removing amoxicillin from wastewater. In addition to supplying dissolved oxygen between 1 and 3.5 mg/L, the value of inlet air provides media rotation. Environmental temperature was between 0 and 31 0C and average pH was 7.2 during this study and effective factors on microorganisms' activity including temperature, dissolved oxygen, pH and other parameters were measured continuously. About three months after reactor's set up, biofilm growth on kaldnes media was observed and following this stage, the reactor with continuous flow for examining the value of amoxicillin removal at different working conditions (4, 6, 8, 10 hours) was applied.

The stages of 8 hour treatment in this system are as follows;

1- At first stage, sewage was feeding into the 100L tank continuously and automatic aeration valve turned on for 6 hours during which sewage automatic outlet valve was off so there was no sewage outlet.

2- At next stage, automatic aeration valve was off automatically and sedimentation continued for 1.5 hours and still outlet automatic valve was off and there was no sewage outlet.

3- At third stage, following 7.5 hours sewage automatic outlet valve was on for 0.5 hour and sewage was drained. This 8-hour cycle was repeated continuously.

Materials and methods

According to national committee for prescribing and rational consumption of drug in FDA of ministry of health, initial standard for most-used antibiotic of amoxicillin in Iran is determined as powder standards and analytical grade with more than 99% analytical purity percentage from company of Sigma Aldrich from USA was applied and all of used solutions include Methanol (HPLC grade), Acetonitrile and two times distilled water with laboratory purity degree were utilized. Balance cartridges (Oasis HLB Cartridges, 500mg, and 6mL) was prepared from company of Milford, USA. Other consumed chemical materials with laboratory purity degree were supplied from Agency of Merck Company of Germany.

Sampling

Sampling was done from inlet and outlet of examined reactor and samples were transferred to laboratory next to 4 0C ice.

Preparation of standard storage amoxicillin solution and sewage samples

In order to preparing stock solution, 500mg powder amoxicillin was took by a digital balance and dissolved in 1000mL distilled water (deionized water). Then concentrations of 5, 10, 15, and 20 mg/L from stock solvent were evaluated and prepared based on relation 1-2.

(1-2) $C_1V_1=C_2V_2$

$C_{1,\ 2}$= stock solvent concentration and desired solution

$V_{1,\ 2}$= volume of stock solution and desired solution

Preparation Method and Sample Analysis

During exploitation of pilot, DO, pH and temperature were measured by multi-probe device from Hach company. Since every antibiotic material has special mobile phase and different analysis conditions, a liquid chromatography device HPLC Agilent 6890n (Agilent, USA) was applied for identifying them separately. Required mobile phase for identifying amoxicillin composition consisted from phase of A: 5% methanol and phase of B: 95% of buffer ammonium acetate. Speed of sample flow in this device was set on 1 mg/min. Since standard sample of amoxicillin was identifiable between wavelength of 230 and 235 nanometer absorbed by UV ray, the peak value of amoxicillin read in the chromatography device was at 9.79 min.

Findings

This experimental study was carried out with the aim of evaluating the process of cyclic activated sludge system with rotating bed in amoxicillin removal from sewage whose results will be elaborated in this chapter.

Note: The efficiency of amoxicillin removal during whole of this text refers to eliminate chemical oxygen demand (COD) of antibiotic amoxicillin.

Statistical Analysis

First stage in analyzing results was to choose a suitable model for this system which is able to predict results with an appropriate accuracy. In order to collect maximum data by the least trials during this study, designing CCD (one of designs from RSM family) for modelling and analyzing trials using software DESIGN EXPERT 7 were applied. Table 1-4 illustrate trial conditions based on response level method in model of CCD.

Table 1. Trial conditions for removing c amoxicillin through cyclic activated sludge system with rotating bed (MBAS)

Run	MLSS (mg/l)	HRT(hr)	Packing Rate(%)
1	2000.00±150	8.00	60.00
2	1000.00±150	12.00	60.00
3	2000.00±150	8.00	40.00
4	3000.00±150	4.00	20.00
5	2000.00±150	8.00	40.00
6	3000.00±150	4.00	60.00
7	2000.00±150	8.00	40.00
8	1000.00±150	8.00	40.00
9	2000.00±150	4.00	40.00
10	2000.00±150	12.00	40.00
11	2000.00±150	8.00	20.00
12	1000.00±150	4.00	60.00
13	3000.00±150	12.00	20.00
14	1000.00±150	12.00	20.00
15	3000.00±150	12.00	60.00
16	2000.00±150	8.00	40.00
17	3000.00±150	8.00	40.00
18	2000.00±150	8.00	40.00
19	1000.00±150	4.00	20.00
20	2000.00±150	8.00	40.00

Designing trials for removing amoxicillin by cyclic activated sludge system with rotating bed (MBAS)

The impact of three major parameters a: percentage of media filling, b: HRT, c: concentration of MLSS (TABLES 2, 3) on efficiency of MBAS system in removing amoxicillin in terms of percentage were examined.

Table 2. Examined variables and range for amoxicillin removal by cyclic activated sludge system with rotating bed (MBAS).

Factors	Symbol	Coded Level -1	0	+1
MLSS Concentration (mg/l)	A	1000±150	2000±150	3000±150
HRT (hr)	B	4	8	12
Packing Rate (%)	C	20	40	60

Table 3. Trial conditions and removal percentage of amoxicillin by cyclic activated sludge system with rotating bed (MBAS).

Run	MLSS (mg/l)	HRT (hr)	Packing Rate (%)	Removal Efficiency Ampicilin (%)
1	2000.00±150	8.00	60.00	75.2
2	1000.00±150	12.00	60.00	71.1
3	2000.00±150	8.00	40.00	48.3
4	3000.00±150	4.00	20.00	33.1
5	2000.00±150	8.00	40.00	50.5
6	3000.00±150	4.00	60.00	82.5
7	2000.00±150	8.00	40.00	48.5
8	1000.00±150	8.00	40.00	43.5
9	2000.00±150	4.00	40.00	40.2
10	2000.00±150	12.00	40.00	58.5
11	2000.00±150	8.00	20.00	30.1
12	1000.00±150	4.00	60.00	62.2
13	3000.00±150	12.00	20.00	55.3
14	1000.00±150	12.00	20.00	30.5
15	3000.00±150	12.00	60.00	94.3
16	2000.00±150	8.00	40.00	50.7
17	3000.00±150	8.00	40.00	69.5
18	2000.00±150	8.00	40.00	48.1
19	1000.00±150	4.00	20.00	25.2

20	2000.00±150	8.00	40.00	46.1

Performance of the process of cyclic activated sludge system with rotating bed (MBAS) for removing amoxicillin

Determining the impact of MLSS concentration, fill percentage and hydraulic retention time upon efficiency of the process of MBAS in amoxicillin removal from wastewater

The results obtained from this stage of trials have been illustrated in table 3 and diagrams (1, 2, 3, 4, 5, 6, 7, 8 and 9) for amoxicillin removal. At this stage, the impact of concentration (150± 1000), 2000±150, 3000±150 MLSS mg/l) on efficiency of amoxicillin removal within retention times of 4, 8 and 12 hours was examined. Mentioned diagram indicated that by increasing retention time and system fill percentage, the efficiency of amoxicillin removal will be increased. Research results showed that suitable designing of the system including aeration, hydraulic retention time, selecting appropriate bed and reactor's fill percentage are very effective in reaching for desirable results [85]. According to obtained results from present study, the rate of system efficiency will be increased at higher concentrations of MLSS and longer retention times. The higher efficiency of system can be accompanied with biofilm growth and increasing number of microorganisms during longer retention times.

Results from present study showed that at 20% of reactor's filling with media, the efficiency of amoxicillin removal at hydraulic retention times of 4, 8 and 12 hours equaled 46.7, 58.4 and 70.1 percent and for 60% filling, this efficiency was 67.8, 79.5 and 91.3 percent respectively.

The efficiency of treatment in biological system relies on the level of availability for biofilm growth and in turn it depends on characteristics and filling percentage of reactor with media which can have a significant impact on biological activity of microbes in the bioreactor [87]. Therefore, increasing filling percentage will raise the surface for growing biofilm. In a study by Feng et al. in 2000, some similar results were obtained for removing organic compounds and Nitrogen by MBBR system [87] (100).

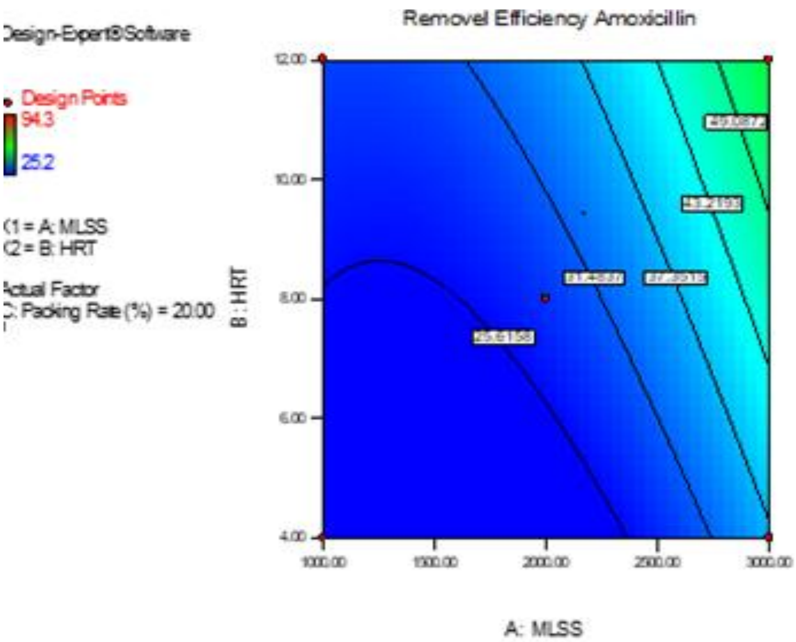

Diagram 1. The desirability percentage of health indicators for tools and equipment in bakeries across the city of Darrehshahr in 2020.

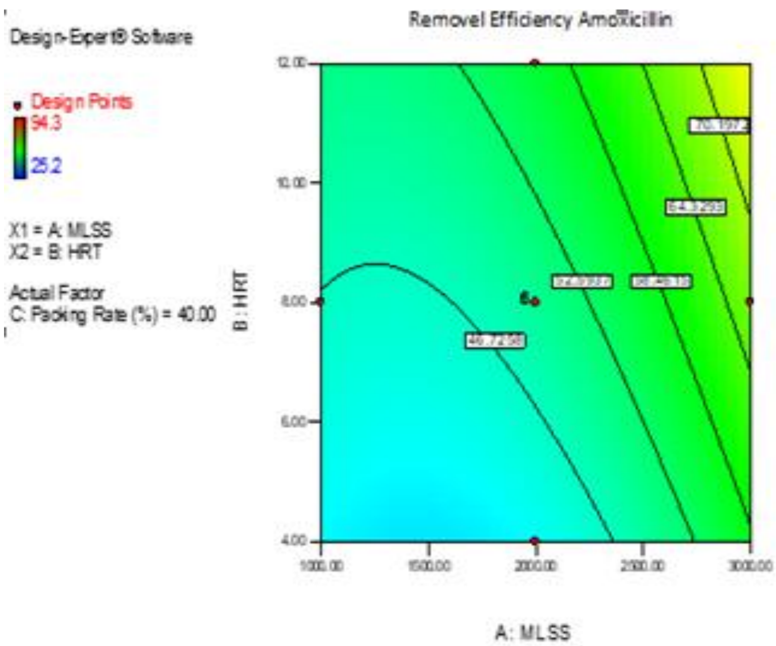

Diagram 2. The efficiency of amoxicillin removal with filling percentage of 40% by MBAS system

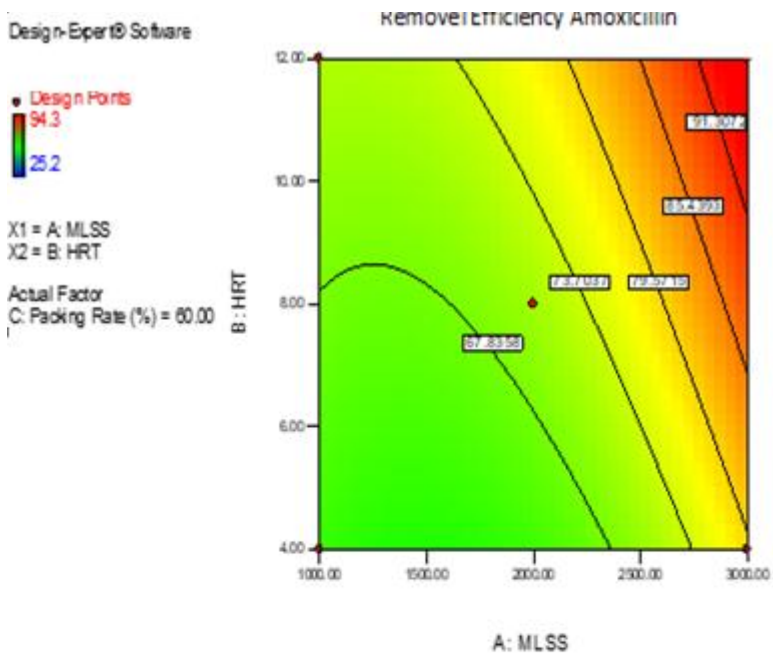

Diagram 3. The efficiency of amoxicillin removal with filling percentage of 60% by MBAS system

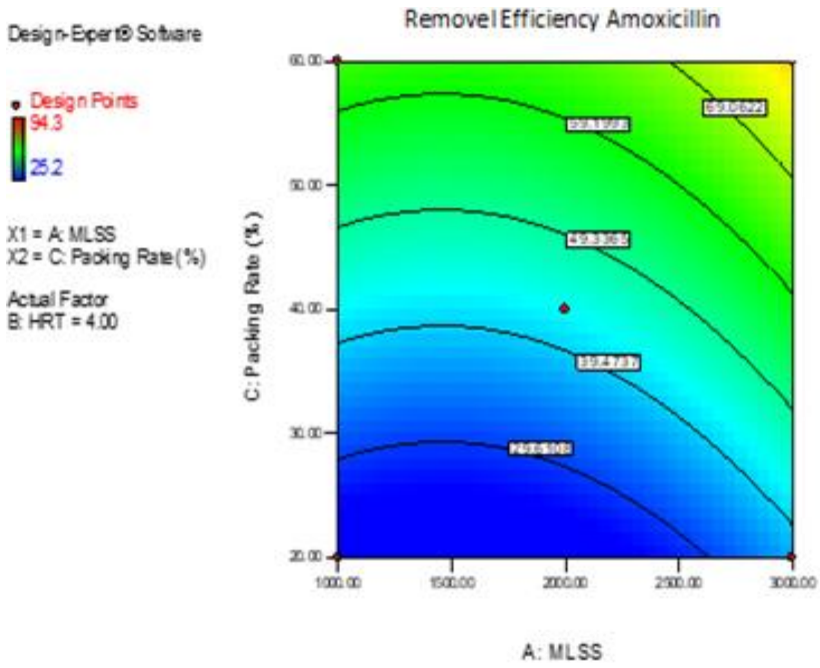

Diagram 4. The efficiency of amoxicillin removal with 4-hour hydraulic retention time by MBAS system

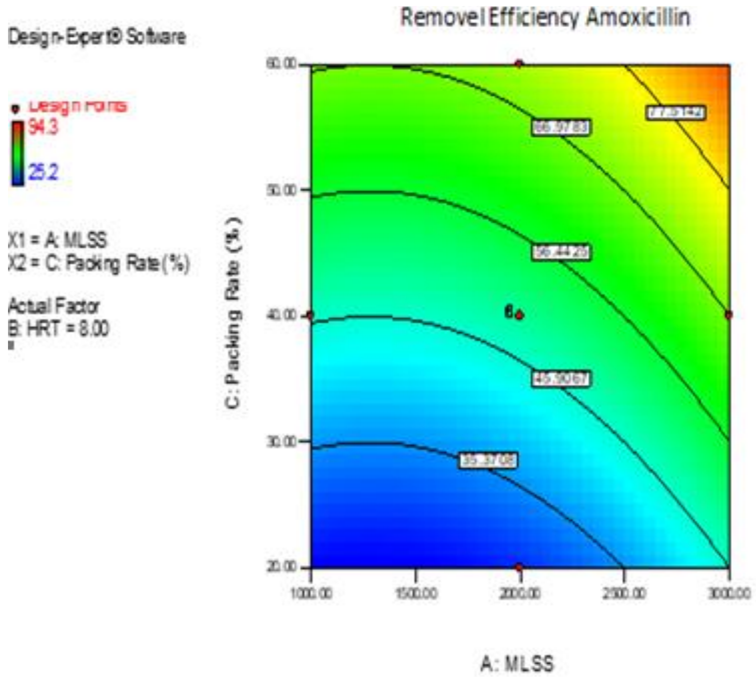

Diagram 5. The efficiency of amoxicillin removal with 8-hours hydraulic retention time by MBAS system

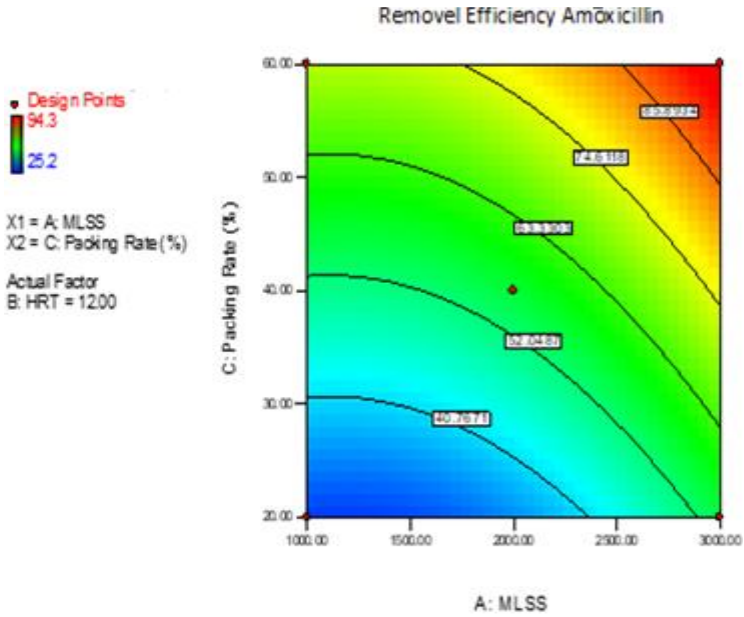

Diagram 6. The efficiency of amoxicillin removal with 12-hours hydraulic retention time by MBBR system

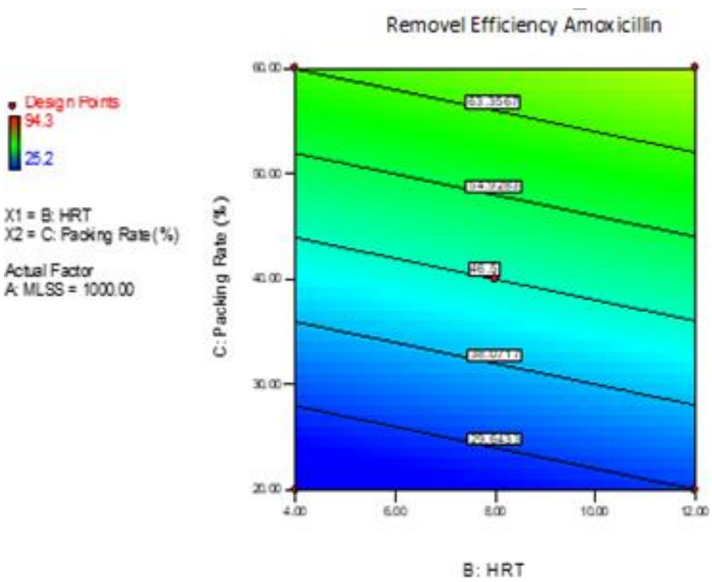

Diagram 7. The efficiency of amoxicillin removal with MLSS concentration of 1000 ± 150 (mg/l) by MBAS system

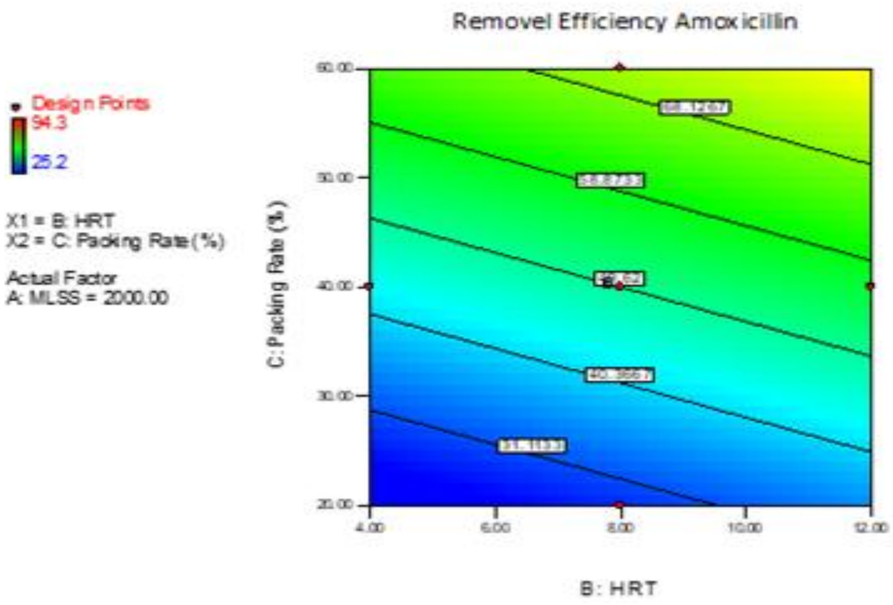

Diagram 8. The efficiency of amoxicillin removal with MLSS concentration of 2000 ± 150 (mg/l) by MBAS system

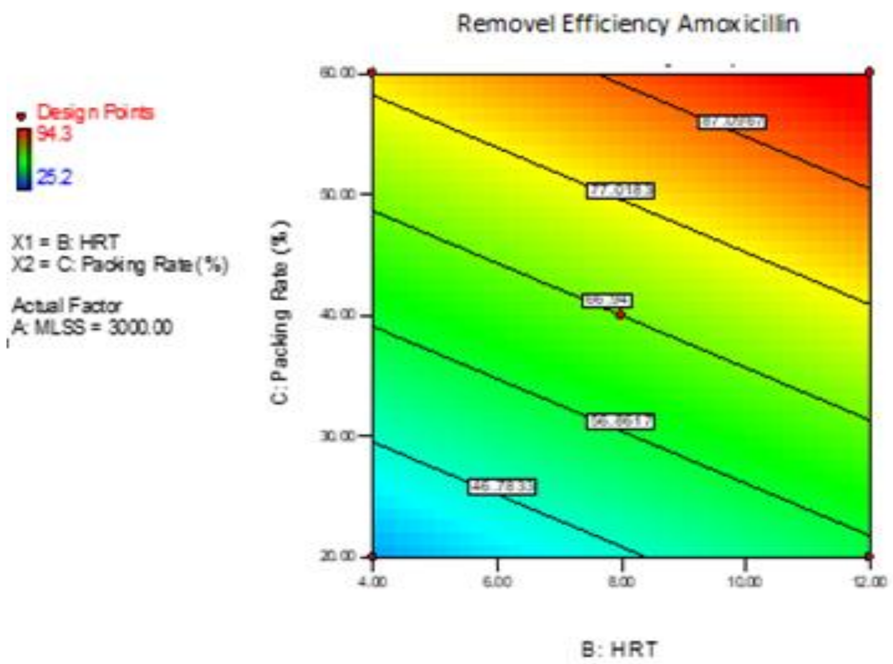

Diagram 9. The efficiency of amoxicillin removal with MLSS concentration of 3000 ± 150 (mg/l) by MBAS system

Discussion and Conclusions

Removing amoxicillin by cyclic activated sludge system with rotating bed (MBAS)

Following determining optimal conditions by MBAS process, optimal concentration of amoxicillin was injected to the biofilm reactor system with rotating bed. Obtained results from MBAS system during this study indicated that so called system can be completely successful in terms of removing amoxicillin. Obtained results showed that compatibility period was very short in this system and it can be exploited soon. Respecting starting work at hydraulic retention time of 4 hours, the biofilm grew slightly later that one of reasons was high value of dissolved oxygen which caused creating shear layer and peeling of biofilm. Media at this retention time were floating at the surface due to its lightness. Along with reduction of dissolved oxygen at retention time of 8 hours, this system reached to a stable state and biofilm layer was composed, hence the efficiency was increased significantly so that media was immersed at the depth due to its high weight, therefore the efficiency was increased due to rising the value of biological mass.

In a study by W Go, H.Hong, by applying polyurethane media in inlet COD, the removal efficiency of phosphorous was 100 percent [101]. In a study on garbage leachate with inlet COD of 100, the removal efficiency of COD was 91 percent. The acnes were made of PVC tubes [102]. By comparing obtained results from this study with other studies, it was appeared that due to type of sewage composition (industrial or urban) and the rate of inlet concentration, reactor's filling percentage, type of applied media and environmental conditions of pilot in other researches which were effective factors on MBAS system and had influence upon system efficiency, different results were achieved. The results of present study indicated that by increasing hydraulic retention time and bioreactor's filling percentage with media and raising MLSS concentration, the removal efficiency of amoxicillin would be increased.

Suggestion for further research

As every research can underpin scientific progress and enlighten many unknowns, it can offer other research subjects in exposing to several issues and problems during research stages and realizing insufficient understanding about field of research. On the other hand, every research is regarded as a guide for further studies and more complete examination of issues' aspects, therefore it is suggested to consider the following subjects for further research:

Respecting results from present study, biofilm reactor system with rotating bed (MBAS) in removing amoxicillin from aqueous environments is suggested. For more antibiotic removal from aqueous environment with higher concentrations or higher efficiency, it is suggested to other researchers to examine longer retention times.

Practical suggestions

It is suggested to monitor and measure antibiotics in hospital wastewater treatment plants based on routine pattern.

It is suggested to monitor and measure antibiotics in urban wastewater treatment plants based on a routine pattern.

It is suggested to monitor and measure antibiotics in a routine pattern at water reservoirs.

It is suggested to monitor and measure antibiotics at water treatment plants based on a routine pattern.

It is suggested to monitor and measure antibiotics in rivers based on a routine pattern.

It is suggested to study antibiotic removal in water reservoirs at actual conditions.

Ethics approval

The present research project was reviewed and approved by Ilam University of Medical Sciences with the research approval code 914016.179.

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Conflict of interest

The authors report no conflict of interest in this study.

Authors' contributions

Data gathering, analyses and interpretation done by Kazembeigi F, Nabi Bid Hendi G, Mousavi G drafting of the paper were done by All authors. Mehrdadi N, and Kazembeige F reviewed the literature. All authors read, revised, and approved the final manuscript.

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