Can Induced Magnetic Field Enhance Bioprocesses? - Review

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Abstract. This review presents a compilation of works with particular interest in the application of static magnetic field (SMF) to biological systems, wastewater treatment and few available reports on microbial granulation technology. It also highlights the effects of SMF on biological systems and wastewater treatment process. With an increasing need for environmentally conscious solutions to water purification and disinfection, wastewater treatment, bioremediation and other cheap alternative means, the application of SMF in biological water and wastewater treatment without increase in chemicals required may become an attractive option. Application of SMF has been reported to be successful in a number of fields including treatment of wastewater. However, there are sparse reports on SMF application in the formation and development of microbial granule and production of extracellular polymeric substances (EPS). Achieving a short start-up time in a bioreactor towards the development of microbial granule is of paramount importance in granulation technology. Ascertaining how effective varying strength of SMF and other input variables may enhance the microbial granule with respect to its physical, chemical and biological characteristics requires further research.

1 Introduction

One important advancement and breakthrough in biological wastewater technology is the introduction of high-rate reactors; in which biomass and liquid retention are in the same basin and thus reduces space required e.g. sequencing batch reactor (SBR). In addition, the process microbial granules are very unlike the conventional microbial flocs. They have denser and stronger aggregate structure; higher biomass concentration, greater ability in withstanding shock loadings and better settleability and these ensure solid effluent separation with ease [1-3].

Microbial granules are categorized basically as aerobic and anaerobic granules. They could be formed through sequential self-immobilization of microorganisms. The development of anaerobic granule has been studied in detail using upflow anaerobic sludge blanket (UASB) having similar regimes [4].

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The performance of UASB reactors in terms of removal of organic matter from municipal and/or industrial wastewater has been reported [5]. Aerobic microbial granulation has been reported as well. They are known to develop faster and effectively under desirable conditions [6]. Aerobic granulation could be carried out mainly in sequencing batch reactors (SBRs) [7]. It has been used for the treatment of low to high strength wastewater containing organics, phosphorus, nitrogen, and toxic substances contained in wastewater [8-9].

Granulation technology is known to be one of the most promising treatment processes. The success of microbial granulation in the treatment of wastewater has been reported [10-11]. Well documented review on trends in aerobic and anaerobic granulation [5,12-13] and the physicochemical characteristics of microbial granule [14] have been also reported.

In order to aggregate bacteria and subsequently develop granules, some vital conditions would be required to be fulfilled [10]. Attempts to reduce the start-up time has led to an increase in the volumetric loading rate of the reactor [15], applying sludge washout strategy [16], trying suitable inoculation sludge and a variety of bioreactors [17]. Also, the nature of substrate composition, hydrodynamic shear force, hydraulic retention time, settling time, aerobic starvation, presence of calcium and other ions, intermittent feeding strategy, dissolved oxygen, pH, temperature, seed sludge selection, reactor configuration, presence of inhibitory compounds, amongst others are factors that could affect the time needed for the biogranule development. Aside these strategies a likely promising alternative is the application of static magnetic field (SMF) to biological wastewater treatment so as to accelerate the biological activity. This is possible by exposing the bioreactor to a created magnetic field during the treatment process. All organisms are generally said to possess internal magnetism. The magnetic field inside organisms known as magnetic biologic effect can bring about metabolism of the living organism [18]. The magnetic biologic effect has been applied in wastewater treatment and its effect on microorganism has been reported [18-21]. The biologic effect in organisms has a relationship with the biomass metabolism, cell membrane permeability and enzyme activity, etc. Although the physiology of magnetic biologic effect on the microorganisms is not completely understood, some investigations have indicated that additional magnetic field is a promising approach in the enhancement of biological activity. Some factors have been identified to stimulate bacteria to secrete more EPS such as organic loading, hydrodynamic shear force, substrate composition, hydraulic retention time, substrate composition and settling time amongst others have been demonstrated to stimulate bacteria to secrete more EPS. [22-23]. This is similar to factors which could reduce bioreactor start-up time as mentioned before. There may be need to further elucidate on the effect of SMF on the granulation process. However, one study observed that SMF could improve granule development and also enhance the EPS production [24].

The operating conditions of a reactor such as organic loading rate, solids and hydraulic retention times, etc., and probably the wastewater composition can affect the eventual granular anaerobic or aerobic sludge characteristics. To achieve a stable operation of the reactor, appropriate design of the reactor based on the expected granular sludge characteristics would be required to be done. In addition, it is important to highlight the bioaggregation process and the development of the microbial granule. More so, it is important to mention reported external effects such as the magnetic field presence and its potential magnetic field intensity range (milli Tesla (mT) to Tesla (T)) in biological systems. On another hand, whether it is an anaerobic or aerobic process, some drawbacks exist. The slow growth of microorganisms in the anaerobic process is the major drawback in the operation of the anaerobic granular reactor as the start-up time of the reactor is pretty much longer, but it is followed by a later rapid development of anaerobic granules [25]. In the case of aerobic granular sludge reactors, the limiting factors for scale up of the process includes effluent high suspended solids (SS) and relatively high cost associated with

aeration [26-27]. Therefore, a better understanding of the process and implementation of sustainable techniques such as the application of SMF around the granular sludge reactor to stimulate and enhance the production of microbial granules, improve the physico-chemical characteristics and EPS formation and production is required. In addition, it is an effort to reduce energy requirements in aeration phase and the overall time required for microbial granule development.

The purpose of this mini-review is to stimulate further ideas which would lead to fundamental research to better understanding of SMF application and possible effects in microbial granule development. The article categorizes the SMF strength levels, mechanism action and effects, and also presents the behaviour of biological systems under the influence of SMF and during its application in biological wastewater treatment. Recommendations for possible future work are highlighted.

2 Static Magnetic Field (SMF)

The effect of SMF application on biological systems and wastewater treatment process has been of interest to some researchers. To the best of our knowledge, the application of SMF in microbial granule development has not been fully explored nor understood. It may have been widely applied in the field of medicine and related medical fields, microbiology, applied science and engineering, but its direct application in biological wastewater treatment still requires further exploration. A difference in the nature of exposure and condition of the environment has been reported to make evaluation of SMF on biological systems or wastewater treatment a bit complicated. Three categories of SMF have been reported. This includes, weak static magnetic field (< 1 mT), moderate intensity static magnetic field (1 mT to 1 T) and strong and ultra strong static magnetic field (1–5 T for strong and > 5 T for ultra strong) [28]. The success of SMF application in biological treatment of wastewater via developed granules has been reported [24]. In as much as the treatment performance appear to be satisfactory, to understand the mechanism and effect of SMF on biological systems and treatment processes, how the strength of SMF affects a system is very important.

2.1 Magnetic Fields Applied in Experimental Systems

The magnetic fields used in experimental systems maybe generated by electric currents in conductors or permanent magnets. These fields are known vector quantities because they have a magnitude described as the field strength (H). The relationship which occurs due to the interaction of the magnetic field with the surrounding medium is the magnetic induction (B). This is related to the field strength by the magnetic permeability (μ) of the medium as in Eq. (1).

$$B = \mu H \quad (1)$$

Whereas in a vacuum (for air compartments), where $\mu = 1$, the magnetic induction usually measured in Gauss (G) is numerically equal to the magnetic field strength which is measured in Oersteds. In practice, the magnetic field strength can vary from one point to another inside a bioreactor treatment system or chamber. This variation is also described by another vector known as the gradient of the field (∇H) and the units of measurement is Oe/cm or G/cm.

Magnetic fields maybe distinguished from each other based on the nature of the source generating the field strength. Static magnetic fields are known to have a constant strength over time and are called homogeneous field. They can be easily produced by permanent magnets or direct current (DC) electromagnets as compared to the oscillatory or time-varying magnetic fields usually produced by alternating current (AC) electromagnets. Thus

the magnetic field is known as a heterogeneous field. In most microbial granule development studies, homogeneous magnetic field has been employed. Permanent magnets are highly recommended because of their relatively lower costs, stand alone nature and ease of maintenance. Desired strength of magnetic fields may be obtained by varying the distance between poles or magnets as the case maybe. Another method to produce stable magnetic field is to use electromagnets. An electromagnet is a pair of Helmholtz coils which are coaxial with either an iron ore or air. Where the coils are separated by equal distance to the radius of the coil, a homogeneous field is sure to exist. The sole advantage of this method is that the magnetic field strength can be easily varied by an increase or decrease in the current delivered to the coil. The disadvantage of this method is the additional energy cost that could be incurred [29].

2.2 Effects of Static Magnetic Field on Biological Systems

There are a lot of areas where the bioeffects of SMF have been studied. A study centered on ascertaining if people living or working around SMF facilities or electrical power lines could be prone to cancer [30]. Several studies have also reported on cells [31], tissues [32] living organisms [33], viability and proliferation [34], activity of enzymes [35], transport of ions [36], gene transcription [37] and bacteria [38-40]. This review also presents how SMF affects growth of microorganisms (bacteria), morphology, enzyme activity, gene transcription and DNA amongst others. The use of bacteria strains for biodegradation in wastewater treatment cannot be over emphasized. In particular, their singular unique characteristic of surviving at about 37°C has made it become a veritable means to degrade a wide variety of contaminants in wastewater.

2.3 Effect of Static Magnetic Field on Bacteria Viability, Growth and Morphology

The bioeffects due to the application of SMF in biological systems and processes have shown diversified results in many studies. A study was conducted comparing the biological effects of SMF on bacteria *Escherichia coli*, *Leclercia adecarboxylata* and *Staphylococcus aureus*. These three different bacteria strains were exposed to the SMF ($t < 30$ min, $B_m = 10$ mT, $f = 50$ Hz) so as to carry out a comparison in terms of viability (number of colony-forming units (CFU)) under such SMF influence. A measurement of CFU with respect to time of exposure was done. The findings show that the quantity of the effect was strain-dependent. Although there was a general decrease in the CFU when the SMF was switched on, the maximum reduction in quantity and highest SMF effect was observed in *E. coli* whereas the smallest appeared to be on *Staphylococcus aureus*. These results were further validated using a t-test [41]. Changes in the morphology of bacteria due to effect of SMF have also been reported. *Escherichia coli* (rod like) and *Paracoccus denitificans* (spherical) were induced by 50 Hz 10 mT. After 1 h SMF exposure, there was no observed change in the bacterial morphology in terms of surface and shape. In addition, the bacteria remained alive after the experiment [42]. This was also in agreement with another finding [43]. Study of the effect of SMF on bacteria under anaerobic and aerobic condition has been conducted. Bacteria growth rate and population were the main focus. Under the anaerobic condition, the study found that there was decrease in the growth rate and number of bacteria for *S. mutans* and *S. aureus*, while under the aerobic condition, their growth was improved. There was however no growth effects on *E. coli*. It could be suggested that oxygen availability facilitated growth of the bacteria *S. mutans* and *S. aureus* [44]. Application of 50 Hz SMF

to the growth of yeast *Saccharomyces cerevisiae* has been reported. Induced SMF up to 10 mT were exposed for 24 min inside a room with temperature of 24-26°C and an air ventilator system to assist maintain the laboratory temperature. Measurement of the yeasts in broth was done and CFU was enumerated. SMF was found to decrease the number of yeast, and at the same time, it slowed down the growth [45]. Various SMF ranging from 10 – 50 mT were applied to activated sludge in a bioreactor for wastewater treatment. The effects of the various SMF on the activity of activated sludge were assessed at a characteristic low temperature of 5°C. The TTC dehydrogenase activity (TTC-DHA) had the highest concentration at 40 MT. Bacteria was found to adapt better at 30 mT. Between 20 to 40 mT, microorganisms produced more unsaturated fatty acids thereby stimulating TTC-DHA which eventually adapted to low temperature. SMF intensities from 1.31-20 mT were optimum for strengthening the activity and viability of the activated sludge microorganisms in the biological wastewater treatment under a very low temperature [46]. Increase in the SMF strength from 16.8-95 mT yielded an enrichment of some strains (*Planctomycetales*). This indicated that SMF was able to enhance the growth of bacteria in addition to the fast start-up of the anaerobic biological treatment process [47].

Magnetic materials have been added in composites to assist in protein separation in biosuspensions. This has made their use in biotechnology applications become of importance. The recovery of proteins also depended on their ion-exchange binding mechanisms [48]. Magnetic particle and magnetic nanoparticles applications in medical field have been described elsewhere [49]. Because of their physical and chemical properties, they have been very useful in biological and environmental applications and they continue to be in high demand. The only backdrop is that they find their way to the environment and their consequent potential health effects on human health. This will need to be addressed by the research community [50]. While there are concerns in different environmental compartments such as soil, water and air, and likely impact on human health, their performance and final fate in sludge after use in wastewater treatment process requires attention [51].

2.4 Effect of Static Magnetic Field on Enzyme Activity, Gene Transcription and DNA

Moderate SMF applied to different densities of *Escherichia coli* and *Pseudomonas putida* gave some interesting findings. Since, literatures reported the increase in efficiency when SMF was applied; it was therefore not out of place to study SMF effect on growth and enzymatic activities during a treatment process. Thus the findings showed that SMF (17 mT) negatively influenced growth, but positively affected the enzymatic activities and the Adenosine 5'-Triphosphate (ATP) levels. The SMF effect was optimum at temperatures 37 and 28°C for *E. coli* and *P. putida*, respectively. The negative effective on the growth of *E. coli* and *P. putida* was most obvious after temperatures 37°C and 28°C, respectively. The results were reversible after the SMF were removed. On another hand, the growth of the bacteria did not improve significantly when SMF strength was 5 mT and 50 mT. The SMF of 17 mT was found to increase dehydrogenase activity and ATP concentrations. It also increased the expression of the *rpoS* gene in *E. coli* [52]. An external magnetic field modified bacteria *Flavobacterium* sp. and only about 50% of enzyme activity was possible after 6, 16.6 and 23.4 h of incubation at 55°C. Also, the pH stability and localized resistance of the bacteria under alkaline and acidic conditions were observed to increase. The outcome of this study showed the negative effect of various magnetic field on the enzyme activity and its thermal stability in the presence of magnetic Fe₃O₄ nanoparticles [53]. Low temperatures of 5°C may affect microorganism enzymatic activity under the influence of

SMF [54]. Cellular stress patterns during SMF exposure have been a controversial area of study. In a related work, the effect of 1 mT, 50Hz on heat shock protein synthesis (HSP) in *E. coli* was evaluated. Comparison was made between SMF (sinusoidal) pulsed square wave (PMF) types of magnetic fields. It was observed that the SMF had a significantly higher level of Dnak and GroEL proteins compared to the PMF. Also, SMF and PMF yielded an increase and decrease in HSP quantity in comparison to the control, respectively. However, SMF affects the synthesis of HSPs in *E. coli* but in a manner that it depends on the signal characteristics [55]. The findings in this particular study is consistent with some other researchers reports especially with respect to biological models exposed for short duration of 30 to 60 min and at 50/60 Hz SMF exposure of low magnetic flux density (8-2300 μ T) [97-100]. In terms of SMF effect on the changes in gene transcription, some works have been reported. A nuclear run-off assay was used to assess changes in specific gene transcription in CEM-CM3 T-lymphoblastoid cells exposed for 15-120 min (and applied 1 gauss sinusoidal magnetic field at 60 Hz). The study considered the changes with respect to time and cell density in the transcription of c-fos, c-jun, c-myc and protein kinase C. In addition, changes in transcript levels, assessed by slot-blot analysis, were found to be parallel to the changes in gene transcription. This finding suggested that SMF had a role in changing cellular processes [56]. Another studied went on to verify this finding and found it was significant ($p>0.05$) and hence proven to be accurate [57].

3 Microbial Granule Development

The microbial granule is a compact and dense microbial aggregate or it is a kind of granular sludge that is formed through microbial self-immobilization process that encompasses physical, biological and chemical phases [10]. There are two types of biogranulation namely anaerobic and aerobic granulation. Aerobic granulation is a biological evolution process from fluffy flocs to compact granules in the presence of sufficient oxygen whereas in the anaerobic granulation oxygen is not supplied in the reactor. Granulation process requires an aggregation of microorganisms that may be affected by the operating conditions in a reactor. Granules consist of millions of microorganisms with different bacterial species that clump together with anaerobic microorganisms in the inner layer of granules and aerobic microbes at outer layer [58]. The use of upflow anaerobic sludge blanket (UASB) reactors for development of granules to treat industrial wastewater has been reported [4]. This has been described as a typical innovative reactor [59-60] biofilm airlift reactors (BARs) [61], and anaerobic sequencing batch reactor (ASBR) [62]. Following this, application of anaerobic granulation technology for wastewater treatment was possible [63]. The anaerobic granules are characterized by high density and compact structure, which can reduce settling time and efficiently improve the separation of treated effluent from the biomass. Anaerobic granules can also maintain very high number of microorganism in the reactor and in turn suitable for high strength wastewater treatment and large volumes of wastewater can be treated in a compact reactor. Anaerobic granule development has some drawbacks, such as very long start-up period, elevated temperatures and not suitability for low strength organic wastewater. Further, anaerobic granulation is unsuitable for N and P removal from the wastewater being treated. As a result of this weakness in the anaerobic development of granules, research is focused towards the development of aerobic granules.

Development of aerobic granule was studied in an aerobic up-flow sludge blanket reactor (AUSB) [64]. Based on this experiment, it was hypothesized that the formation of aerobic granule is similar to the anaerobic granule as filamentous bacteria come together to form granules. In another study, development of aerobic granules using SBR was performed and the very first patent was granted to Heijnen and van Loosdrecht [65] Later, aerobic

granules were developed without support material and the study clearly explained the development stages of the aerobic granule [2]. After inoculation, the reactor system was found to be dominated by bacteria and fungi. Fungi will form mycelial pellets, which settle quickly and are retained in the reactor. Aeration process causes shear force in the reactor, the filaments then detach from the surface of the pellets and the pellets become more compact. The pellets can grow to as large as 5-6 mm in diameter. After which, the pellets will lyse as a result of oxygen limitation in the inner part of the pellets. These mycelial pellets will be immobilized and then grow to form colonies. Thereafter, the bacterial colonies can maintain themselves because they are large enough to settle at fast speed. Thus, the bacterial colonies will further grow to become granules as shown in Fig. 1.

Fig.1. Aerobic granule developed without carrier material [2]

Aerobic granules are compact, regular and smooth rounded shapes that are formed through self-immobilization of microorganisms. During the first aerobic granular workshop by International Water Association (IWA) which was held in Munich, Germany in 2004, aerobic granulation was properly defined. It was defined as "aerobic granular activated sludge as aggregates of microbial origin, which do not coagulate under reduced hydrodynamic shear, and which settles significantly faster than activated sludge flocs". In 2006, the second aerobic granular workshop highlighted the aggregation of microbial origin, coagulation under reduced hydrodynamic shear, settling faster than activated sludge flocs, minimum size, and method of harvesting. When an aggregate fulfils all of these characteristics as described above, it can be called aerobic granular sludge [12, 65].

Some studies observed that both aerobic and anaerobic biogranules have different characteristics and have their respective advantages and disadvantages. The comparison between the characteristics of aerobic and anaerobic granulation process is shown in Table 1. Granulation technology for wastewater treatment offer more advantages than the conventional wastewater treatment especially when aerobic granulation is used. Since early 2000, aerobic granular sludge system became very attractive for treatment of wastewater stemming from its characteristic dense and compact microbial structure, excellent settleability, high biomass retention, ability to withstand high organic loading rates, simultaneous nitrification denitrification, low investment, etc [8, 10, 66-67]. Due to this advantage, aerobic granulation has been used in various type of wastewater treatment including organics, phosphorus, nitrogen and toxic substances removal [68-73], dyes [74-76]. Aerobic granules have also been used in degradation of phenol and pentachlorophenol [77-80].

Table 1. Comparison between aerobic and anaerobic granulation process [81]

Process	Anaerobic granulation	Aerobic granulation
Bacteria characteristic	Do not rely on oxygen for metabolic process and survival	Require oxygen to survive and grow
Operating	Anaerobic (0 mg/L DO concentration)	Anaerobic and aerobic (saturated DO concentration)
Upflow velocity	0.6-2.0 m/h	Higher than 43 m/h (1.2 cm/s) (202)
Biomass concentration	5-40 g/L (top) & 50-100 g/L (bottom)	5-15 g/L
Substrate degradation	Not completely degrade the influent waste. e.g textile wastewater	Complete degrade to the end products. e.g textile wastewater
Formation	Mostly on UASB reactor	Mostly on SBR reactor
Reactor start-up	About 3 month	About 1 month
Operating temperature	Preferable at mesophilic and thermophilic temperature range	Stable at 8-12 °C (8)
Effluent suspended solid	30-150 mg/L	80-100 mg/L
Wastewater strength	High strength wastewater	Low to high strength wastewater
Nutrient removal	Low	High
Simultaneous nitrification denitrification	Impossible	High

Reported works observed that aerobic granules can be easily developed in sequencing batch reactor (SBR) because this system fulfills most of the needed requirement especially operational strategies that contribute a feast-famine model in microorganisms' growth [82]. Development of aerobic granules from various types of seed sludge such as in conventional activated sludge [83-89], autotrophic nitrifying sludge [90], anaerobic granule [73, 91] mixture of activated sludge and anaerobic granules [67], mixture of crushed granules and floc sludge (92-93 amongst others is possible.

Until now, activated sludge has been used as seed in most aerobic granulation studies. Jang et al. [86] cultivated aerobic granules by inoculating sludge collected from a conventional municipal wastewater treatment plant. After 40 d of operation, the seed sludge in the reactor was observed to have formed into a granule and after 50 d operation the irregular granules became stable and round shaped. Arrojo et al. [68] cultivated aerobic granules using industrial wastewater. They demonstrated that formation of aerobic granules was a gradual process from the flocculent seeded sludge to compact aggregates, further to granular sludge (2.3 mm) and finally to mature granules (3.5 mm). Cultivation of aerobic granules by seeding autotrophic nitrifying sludge in SBR has been studied (83). The study reported that matured 0.5 mm aerobic granules were cultivated within 43 d. A modified method was used to cultivate aerobic granules with seeding superior mixed flora (SMF) in

which aerobic granules were formed after 19 d [94]. The study finding suggested that granulation process consisted of five phases, i.e. microbe multiplication, floc appearance, floc cohesion, mature floc and aerobic granule phases. SMF was the key reason for the aerobic granules to be shaped into filamentous microbes and this played a crucial role in granulation process. Aerobic granules have also been cultivated by seeding anaerobic granular sludge from UASB reactor [91]. Granular sludge was reportedly used for treatment of textile wastewater under intermittent anaerobic and aerobic reaction phase [74]. The seeding method involves three main stages of disintegration, recombination and eventual growth. After inoculation, the anaerobic granular sludge disintegrated under aerobic condition and formed irregular and small flocs and debris. Then the small flocs and debris recombined under aerobic condition and finally the aerobic granules were formed. The study suggested that the disintegration of the anaerobic sludge might play a role in nucleus formation during the granulation of the aerobic sludge.

3.1 Effects of Static Magnetic Field on Biological Wastewater Treatment and the Developed Microbial Granules

There are a number of areas where SMF has played important roles in environmental waste-treatment including water and wastewater treatment. Some of the studies are highlighted herein.

The application of SMF in biological wastewater treatment has been of interest [17, 24, 95]. This is possible because SMF is probably able to influence the growth of microbes and biodegradation of pollutants. Static magnetic field is reported to be able to enhance bacterial activity and the effect was quite noticed in heterogeneous cultures (96). According to Lebkowska et al. (95), the use of magnetic field is to enhance the microbial activity and also accelerate the biodegradation rate. Interestingly, SMF effect was assessed when 7 mT SMF was applied during activated sludge treatment and biodegradation of industrial wastewater containing urea-formaldehyde resin (97). The exposure of the reactor to SMF enabled formaldehyde removal by 20% with respect to the control sample. It also increased COD removal with an increase in loading. The activated sludge biomass similarly experienced growth during this study. At certain range of SMF intensity, it is believed that it is possible to promote the microbial metabolism and granulation (24). In addition, low SMF intensity could be more suitable for promotion of the microbial growth as against high SMF which could deteriorate the microbial activities. There has been a study related to the influence of SMF on granule formation (24). It was found that the content of extracellular polymeric substances such as proteins and polysaccharides increased up until 25.4% and 33.3%, respectively when exposed to SMF. In addition, SMF assisted the accumulation of iron compounds in the sludge. This is because iron compounds can be magnetized when exposed to SMF. This helped to enlarge the floc size when the iron compounds were assembled together under the influence of SMF [98].

Improvement in wastewater treatment through application of SMF could be an interesting subject to further explore as results from recent studies have shown. SMF can also reduce cost compared to physical and chemical treatments. Besides, SMF may require a very short period to treat wastewater and would not produce contaminants, thus it reduces the quantity of chemical waste which sometimes causes secondary problem as a result of chemical accumulation and needed disposal. Previous studies observed that SMF can enhance activated sludge performance [96, 99-101] increase biodegradation rate [102 – 104] influence the growth of microbes [105-107] and increase EPS production which eventually enhances development of aerobic granules [24]. However, the precise strength of SMF which could be applied to enhance the biological process of wastewater treatment is still not

very certain. Until now, the reported range of SMF used in wastewater treatment is in range 6 to 10000 Millitesla (mT). But for some microorganism, only very low and suitable SMF may accelerate their growth rate, nutrients absorption and utilization [103]. Low SMF is more suitable to promote the growth of microbes while high SMF could deteriorate the activity of microbial communities [24, 108]. The effect of variation in SMF intensity in biological wastewater treatment systems performance has been studied. Most of the studies conducted their experiment with 6 to 9 pH value and temperature 20 to 35°C. Operation of bioreactors under the influence of SMF showed enhancement and an increase in biodegradability of the wastewater. According to Ji et al. [100], the order of effects on the biodegradation rate is $SMF > pH > temperature$ [98].

The effect of SMF may change the physiochemical properties of waste, or prepared laboratory solutions and biological cultures by influencing nucleation and growth, crystallinity, surface tension, viscosity, scaling kinetic and chemical equilibrium [109]. SMF in another report increased the potential of ions such as manganese, calcium, iron and magnesium in enzymes [110]. On the other hand, during enzymatic reaction in the biological process there has always been an unpaired electron; with the influence of SMF it will affect the reaction process by altering the electron spin state of intermediate product. It was noted that SMF could cooperate with moving charges in the cell and will change their velocities [111]. SMF may also create greater ionic charge or extra energy making the charged particles to possibly vibrate excessively and thus cause more particle collision.

Some studies have reported application of magnetic nanoparticles and SMF and their effects in other methods of wastewater treatment. Most of these studies were used to adsorb contaminants and after the adsorption process, the adsorbent was separated from the treated effluent by inducement of a magnetic field being the actual separation process. This separation technique has been reported in oil-bearing effluent, mine effluent, and some new complex processes, for example in magnetic chemistry processes [112]. SMF has also been applied for the adsorption of organic dyes such as acridine orange [113] congo red [114], for removal fluoride and phosphate [115], eutrophication in lake restoration [116] and to accelerate the coagulation of sewage in bio-system [117]. The treated effluent has high efficiency in terms of separation and can easily allow removal of an adsorbent, which can cause any negative effect at the downstream processing phase. Some other studies have also used magnetic particle combined with magnetic field to increase the performance of the system. Ozaki et al. [118] applied SMF to microorganisms immobilized with magnetic particles for phenol biodegradation. Almost 100% of phenol was removed after 40 d operation of the reactor. Jung et al. [119] reported that combination of magnetic substance and the south pole of SMF had an increase of 30% in the biodegradation rate of phenol. Xu and Sun [103], studied the effect of magnetic field on the removal of Cr(VI) during a biological treatment process. Presence of magnetic particle i.e. nanomaterials improved sludge settling as the settling velocity was decreased from 12 to 8.2%. In addition, they stated that the magnetic particles could play an important role in reducing the sludge loss, decreasing the turbidity of outflow water, which may be needed to stabilize and improve the overall treatment efficiency of the system.

At the moment, there is sparse study in the application of SMF for the development of microbial granules. Focusing on the application of SMF in microbial granule development for wastewater treatment should be encouraged. Apart from enabling the enhancement of microbial granule formation [24,110], increasing floc size [98], aggregation of compounds to reduce granulation time required by improving the settling properties of granules and stimulating more EPS production, Lebkowska et al. [95] found that the use of SMF enhanced the microbial activity and accelerated the biodegradation rate. Tomska and Wolny [99] have applied the SMF to activated sludge process and they discovered that SMF can also accelerate nitrification rate. Hattori et al. [98] opined that the flock size of activated

sludge was enlarged and sedimentation was enhanced by application of an external SMF. These authors also noted that the addition of FeCl_3 to the activated sludge with the influence of SMF would be more effective. It was suggested that the sludge might be magnetized by the external SMF and can also affect the growth of microbes by intensifying the bacteria activities [120]. Another study reported that SMF influence could increase the concentration of the extracellular enzymes on the surface of bacteria [101]. Aggregation of iron was reported to decrease granule formation time by enhancing the settling properties of granules while stimulating the secretion of EPS [24]. SMF is believed to stimulate the production of EPS, increase the microbial aggregates and enhance the entire biogranulation process.

4 Conclusions and Future Prospects

Influence of SMF on the development of microbial granules in biogranulation requires more studies. The effects of SMF on start-up time of the bioreactor, aggregation, hydrophobicity/hydrophilicity, surface charge, settleability, stability and dewatering and flocculation ability of microbial granules needs to be ascertained. More importantly, there is still sparse information relating to the effect of SMF on EPS production. Again, SMF is said to affect microorganisms in certain ways especially with respect to their growth phase and this may affect the EPS content in the various phases. Therefore, the relationship between SMF and EPS production require further research and clarification. The question still pending is can SMF be a probable external factor for development of enhanced microbial granules? And where it can what is the likely SMF strength that should be applied for optimal performance. What temperatures would favour the use of SMF? And would oxygen presence actually determine the performance level such as in aerobic process compared to anaerobic process? These and more can only be answered by conducting further research in this direction. With the volume of research done so far, some surprising features of SMF action on biological systems or processes complicate the interpretation of the underlying mechanisms. However, some information is available and can give an idea to those who may be involved in subsequent study. Firstly, distinct effects are observed at relatively low SMFs. Secondly, the simultaneous presence of nanomaterials, SMF and possible alternating SMFs turns out to be optimal for SMF interaction with biological systems or processes. Thirdly, it is probable that biological responses can be detected only with certain levels of SMF at unexpectedly low magnetic field strength and frequencies. These seem to be the available suggestions from previous studies. The wheel may be reinvented by applying SMF in the development of enhanced microbial granule to answer the question raised herein. The feasibility of engineering certain properties into microbial granules by the application of SMF at appropriate intensity may improve wastewater treatment process. This review will be significant in stimulating fundamental research works and address integration of several other parameters including the application of SMF for treatment of a variety of wastewater via microbial granulation. In addition, it would facilitate alternative solutions to meet the technical and economical (such as the continuous aeration costs) setbacks that maybe presently experienced in microbial granulation studies.

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