A systematic literature review of biocarriers: central elements for biofilm formation, organic and nutrients removal in sequencing batch biofilm reactor

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Abstract
Over the past decades, several materials utilized as biocarriers for immobilization of microorganisms have been gaining popularity showing different degrees of effectiveness. However, information concerning their effects on sequencing batch biofilm reactors (SBBR) performance is still lacking. There is currently no single widely acceptable material documented for proper biofilm formation, as most materials cannot achieve satisfactory level. Problems of biocarrier also exists due to the emergence of newer and more complex pollutants. Therefore, resource-efficient and environmentally friendly biocarriers are a call of the hour. This article thus, presents a systematic literature review of existing research articles on the various advances made between 2005-2021 about biocarrier physical properties, and performances with specific focus on their contributions in biofilm formation, nutrients and organic matter removal. This is to establish their role as central elements in biofilm formation. The review further described the operational challenges, mass transfer considerations, and recommendations made during successful utilization of the biocarriers in SBBR systems. Future research areas that may ultimately lead to large-scale commercial application of this biocarriers and will result in sustainable and environmentally friendly solution to the problems created during wastewater treatment have also been identified. This paper suggests that future researchers investigate the efficiency of composite biocarriers. It is believed that information contained in this review will increase readers fundamental knowledge, guide future researchers and be incorporated into future works on experimentally-based studies on biocarriers in SBBR systems.

Keywords: Biocarriers, Biofilm, Granular Activated Carbon (GAC), Sequencing Batch Reactor, Systematic Literature Review, Polyurethane Foam Cubes

1.0 Introduction

Sequencing Batch Reactor (SBR) systems are non-steady-state, variable-capacity and suspended-growth biological wastewater treatment systems that uses the fill and draw activated sludge (AS) system with clarifier and an intermittent aeration mode where almost all metabolic reactions take place with the segregation of solid-liquid in a unit tank through a timed control sequence [1]. It blends both anaerobic and aerobic stages to successfully achieve nitrification, denitrification and phosphorous removal (SNDPR) concurrently [2, 3]. SBR is used to eliminate high strength organic and inorganic pollutants, nutrients and suspended solids (SS) in a single tank [4, 5]. Thus, it has several other applications as highlighted in Fig. 1.

As a biological treatment system, SBR system has the following benefits: (i) require small footprint, single basin operation with no secondary clarifier required, operated automatically with excellent process control possibilities (ii) Simple and flexible in configuration and operation, low installation and operation cost, high tolerance to various loading shocks with good bulking control (iii) Robustness, higher biomass retention, reduced energy consumption, endurance to toxicity, solid retention time (SRT) decoupling from hydraulic retention time (HRT) and simultaneous organics, nitrogen and phosphorus removal [6, 7].

The use of AS-seeded SBR as a bioremediation technique could shed light on increasing the efficiency of treatment [8]. However, it has been hindered by the limitations of weak settling features, restricted scale-up and maintenance, broad footprint requirement, susceptibility to toxic environment and autoxidation, poor extraction efficiency because of biomass production restriction [9], unstable performance, excess sludge production amounting to high sludge volume index. Therefore, there is need to modify the SBR system and make it feasible for a broad range of applications by developing bioreactors with inert biomass immobilization support mediums called the sequencing Batch Biofilm Reactor (SBBR) [4].

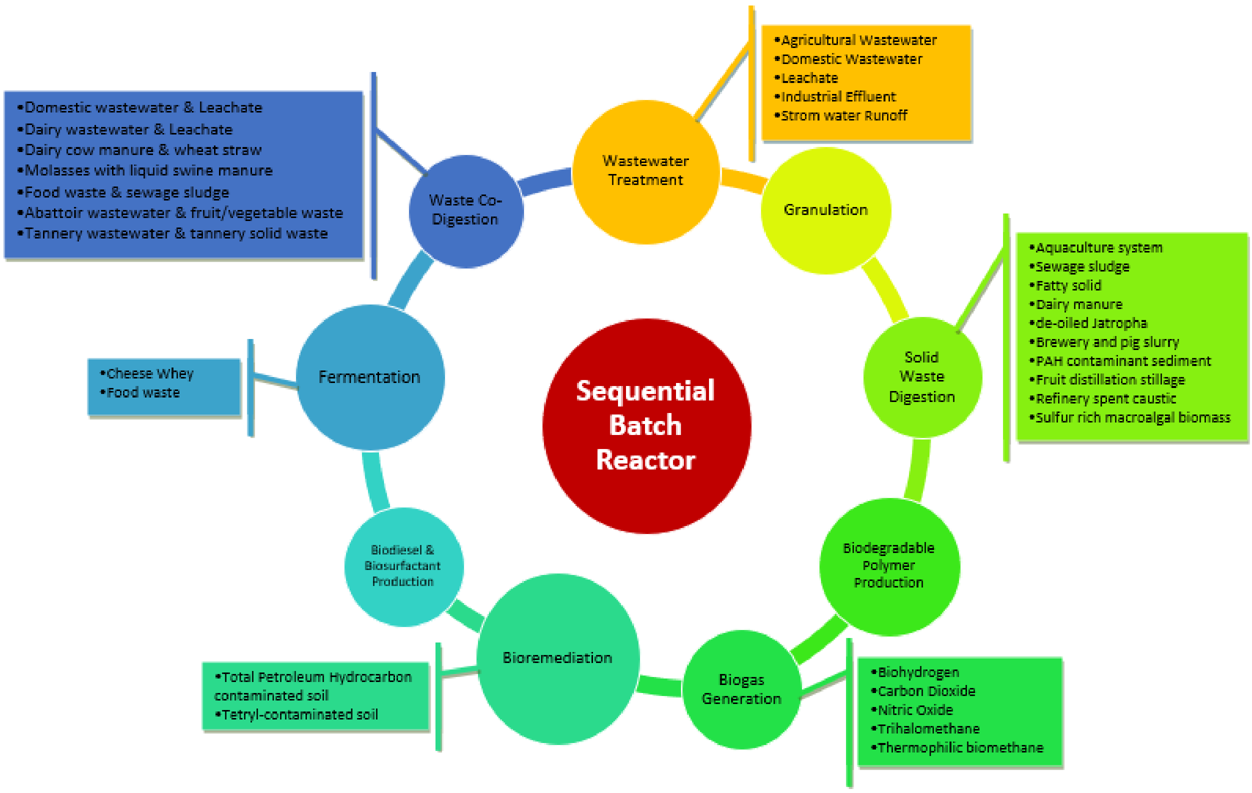


Fig. 1. Sequential batch reactor application for various treatments.

SBBR is a hybrid SBR that suspends AS and connects growth processes into a single biocarrier-filled system where aerobic and anoxic conditions are given simultaneously in the biofilm's inner and outer layers. SBBR could be used for effective biological denitrification and dephosphatation as sufficient biomass can be sustained in the reactor and thus a higher and more stable treatment efficiency achieved, through the use of the biocarrier materials of various nature and types [1]. Finding an optimal operational strategy is a key point to achieve reactor's stability in SBBR. Available literatures have studied parameters such as; influent ammonium concentration, temperature, chemical oxygen demand (COD) load, running pattern, carbon to nitrogen ratio and many others with a conclusion that nutrient removal efficiency in SBR is always lower than in SBBR [10]. In SBBR systems, specific surface area (SSA) is large and the main types of microbial aggregates are biofilm and suspended sludge where biomass grows as a biofilm on small carriers that move freely into the wastewater [11]. The carrier packings serve as filter media to minimize the concentration of SS in the effluent. SBBR system needs no sedimentation tank [12]. An additional biomass inventory is been provided through biomass accumulation on biocarrier media in SBBR. Compared to an SBR system with similar SRT, high biomass concentrations can be attained by an SBBR system, resulting in lower HRT as reported by earlier studies.

Moreover, for the following reasons, SBBR can indeed cope with the heavy hydraulic load: (i) a large number of microorganisms could prosper because of the ideal microenvironment provided by the biocarrier, (ii) The growth of microbial attachment can enhance the degradation of TN, COD, TP and NH_4^+-N (iii) non/intermittent aeration process could promote the activity and growth rate of microorganisms in the biofilm [13, 14].

The application of biocarriers for biofilm formation in SBBR system during wastewater treatment offers the advantages of higher nutrient removal efficiency, rich biodiversity, more compact design, improved sludge quality and biomass retention, energy savings, less footprint, flexible operation and management, higher volumetric loads, risen process resistance to shock loadings, less sludge and sludge conglomeration, good sedimentation, lower sensitivity to toxicity and reduced acclimatization time of the system [15]. More so, startup of the SBBR system could be 2–3 days quicker than the SBR system in attaining steady state [16]. The use of carrier materials to immobilize cells in sequencing batch reactors eliminates uncertainties about sludge granulation, improve solids retention, suppress settling step, reduce total cycle time and consequently increase contact for biological reaction [10]. Relatively, the system experiences fewer bulking problems, minimal clogging in the reactor, large surface area and do not require sludge recycle devices, while still sustaining great microbial assemblies inside the reactor [17, 18].

2.0 Biocarriers and biofilm formation in SBBR

Biofilm reactors are bioreactors that have embedded biocatalysts, either on or attached to the surface of an inert biocarrier, thus forming agglomerates [19]. Situations where there are high differences in hydraulic loading and where gently developing species with unique metabolic functions should be preserved from washout find biofilm reactors very helpful [20]. The bioreactors are capable of providing multiple sub-zones for different kinds of bacteria, thus preventing the slow-growing nitrifying bacteria from wash out in a fight with heterotrophic bacteria [18]. There have been studies of concurrent nitrification, denitrification and phosphorus removal in biofilm processes. Diffusion constraint in the biofilm, however, prevents the removal of biological phosphorus [21]. Irrespective of the kinetics applied in a biofilm system, hydraulic regime has a major effect on the overall process effectiveness of the procedure. Gigantic biomass concentration retention inside the reactor raises the resistance of the process and shock load resistance [22]. The two key parameters that define the efficiency of biofilm reactors are biofilm thickness and density. The thickness of the biofilm is regarded as the distance perpendicular to the biofilm surface substrata from the biofilm-bulk fluid interface. With the biofilm thickness, the biofilm roughness, which influences the fluid movement in the boundary layer, increases. More so, biofilm thickness influences both the useful diffusion coefficients of substrates in biofilms and the mass shift of substrates to the biofilm from the bulk fluid [23].

The diffusivity of oxygen in biofilms for wastewater treatment was studied by Hibiya [24]. The author set forth an empirical equation (See Eq. 1-3) describing the effective distribution coefficient of oxygen, *De* (m^2/s), and the biofilm thickness,

δ (m): $$D_e = (3.5 \text{ x } 10^{-19} + 1.5 \text{ x } 10^{-21}\, \delta)^{0.5} \quad (1)$$

According to the results, with increasing biofilm thickness, oxygen totally diffused toward the biofilms bottom layer and the depth of oxygen penetration increased. Substrates diffusion coefficients via biofilms are significantly influenced by biofilm density, which is the quantity of dry biomass in a given biofilm volume. With declining biofilm density, diffusion transport increases.

De depends on biofilm density, X (g/L), as follows:

$$D_e = 8.154\, x\, 10^{-10}\, x\, 10^{-0.00753X} \quad (2)$$

Thus, an individual column's biofilm density, X' (g/L), is computed using the following equation:

$$X' = \frac{TS}{\delta A_1}(g/TS) \text{ or } X' = \frac{VS}{\delta A_1}(g/VS) \quad (3)$$

where VS (g) is the volatile solids, A_l is the tested columns surface area and TS (g) as the total solids.

The average rates of biofilm density for the three examined columns can be utilized to compute the entire module's X' (g/L) biofilm density [25].

2.1 Biocarriers

A progressively used biological treatment technique is the adhesion of microorganisms on surfaces of biocarriers [16]. The deciding constituent that fixes the maintenance of the potential microorganism and the reactor attachment phenomenon is packing. It is an essential factor for preserving SBBR stability and ensuring internal microbial biomass. [9]. Biocarriers are therefore built to provide the biofilm with a protective surface and optimum conditions for microorganisms breeding while they are suspended freely in wastewater [26]. In order to render surface area for bacterial appendage and growth, biocarriers are inserted into traditional AS reactors. To allow slowly-nitrifying growth and encourage nitrification, biocarriers may also provide greater SRT [27]. Different biocarriers are favorable to the enrichment of diverse and overflowing microorganisms, therefore, the choice of suitable biocarrier is greatly essential to secure the effectiveness of the reactor. The various key factors to be considered during biocarrier design are specific area, thickness of biofilm, biocarrier material (texture, form, etc.), and filling ratio. Research suggests filling ratio to be less than 70% to enable biocarriers move freely [28]. The ability to monitor surface area loading rates through the fill ratio of biocarrier within the bioreactor, is a major advantage of this process. It enables high-rate treatment in a small footprint, making it a cost-effective alternative for treatment [29]. High surface area biocarriers can produce more areas for the growth and absorption of microorganisms. Biocarrier forms and textures have a similar influence on the weight, behavior and structure of the biofilm [30].

In SBBR, microbial attachment and biofilm formation on biocarriers are multiple-step processes. In these processes (see Fig. 2), physicochemical (hydrodynamic, gravity, etc.) and biological (extracellular polymer development, bacteria cluster formation etc.) forces play an important role [31].

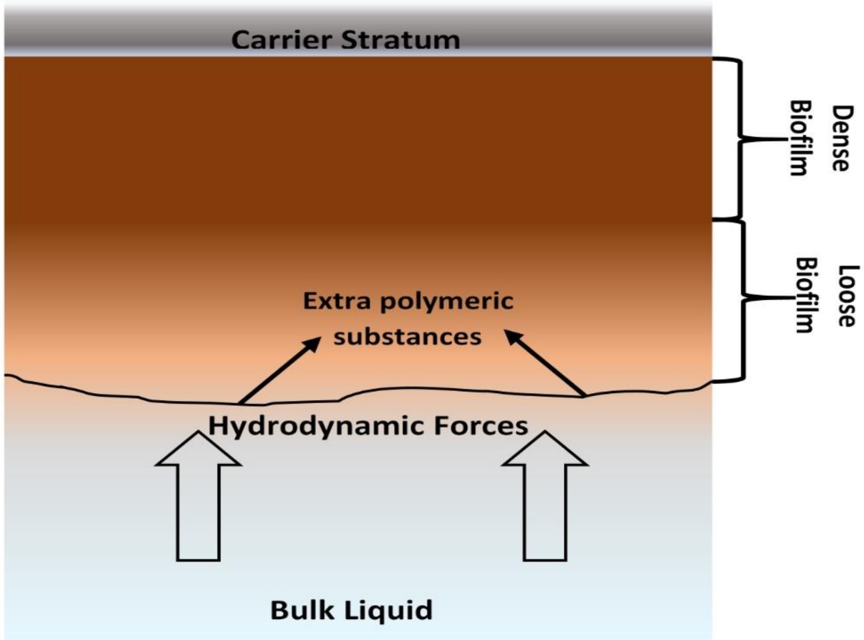


Fig. 2. Multiple-step processes for microbial attachment and biofilm formation on biocarriers

Biocarriers can evenly swim within a moving bed reactor by the turbulent energy supplied by the aerator and mixer [4]. The danger of biomass wash-out can be significantly reduced by immobilizing anammox bacteria as biofilm on the surface of biocarrier. To increase the SSA required for bacterial growth, numerous biocarrier materials have already been used. However, in choosing the most effective one for nutrient removal, DO transport from the bulk solution to the biofilm interior remains the main issue. Daverey et al., [29] in their study also argued that only anammox bacteria could be immobilized by the biocarriers used, while other microbial species (AOB-ammonia oxidizing bacteria and denitrifiers) are also present in completely autotrophic nitrogen removal over nitrite (CANON) and simultaneous nitrification and denitrification (SNAD) systems. This shows that the use of biocarriers involves the biocompatibility of materials with the prevailing microorganism [19].

2.2 Biofilm formation

Biofilm is a tight microbial aggregate formed by means of immobilization of bacteria on the surface of biocarrier, as suspended sludge freely disperses in solution (see Fig. 2). Biofilms are complex 3-D non-uniform bio-structures comprising pores, channels, and irregular protuberance which can produce pleasing anoxic opportunities for denitrifying bacteria [31]. They are created when unicellular organisms and associated deposits come together to form a group that is encased in an exopolysaccharide matrix and attached to a solid surface.

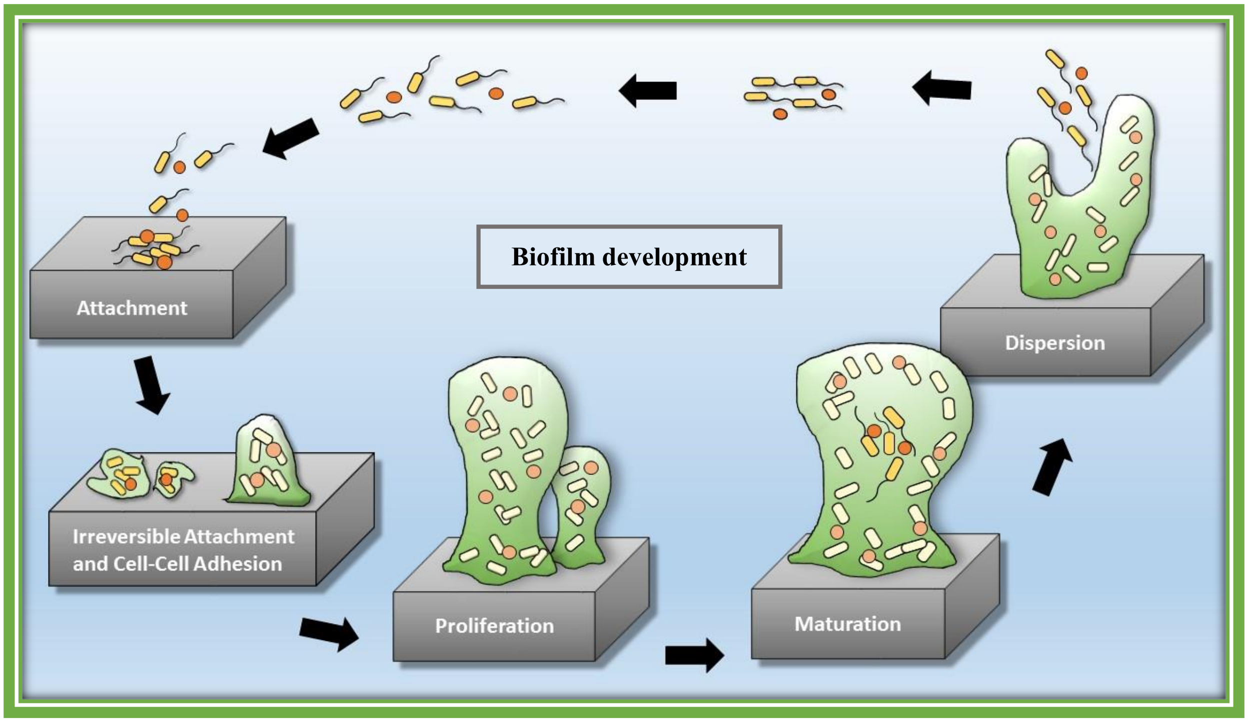


Fig. 3. Stages of biofilm development

Extracellular polymeric substances (EPS), otherwise referred to as slime, are substances of high molecular weight emitted by bacteria that facilitate bacterial adherence to the support media [26]. They are the main biofilm components that significantly connect cells together, and are recommended to increase nanoparticles resistance from AS and biofilm. Due to the rise in mass transfer resistance, their presence will decrease the diffusion rate of toxic elements into the deeper biofilm layer [4]. Thus, EPS can be described as an organic macromolecule emitted by microorganisms adhering to cell surfaces, enhancing bacterial aggregation and promoting the formation of stable structures in the microbial community to further grow biofilms. It is also a simple way to preserve functional bacteria especially in SBR systems.

Biofilm formation on biocarriers is the basis of a successful start-up that plays a significant role in biofilm reactor efficiency. The difference in growth conditions and substrate loadings can lead to differential biofilm growth in reactor tanks. In summary, biofilms development and growth exhibit four stages, namely: (i) initial attachment and young biofilm formation, (ii) biofilms accumulation, (iii) biofilm sloughing and updating, and (iv) biofilm maturation. The morphological structure of biofilm could cause aerobic, anoxic and anaerobic zones to occur simultaneously in the profile of the biofilm. However, variables such as antibiotics, heavy metals, salinity, and nutrient content may affect its role in nutrients and organic matters removal [32]. EPS is specifically correlated to microbial adhesion, aggregation and biofilm formation in SBBR systems. Therefore, analyzing EPS variations can help in understanding biofilm formation [33].

3.0 Research methodology

A systematic review of literature is an accountable, explicit and comprehensive method of study carried out through a consistent and specified process to promote article selection and the screening of relevant review articles. It is a kind of secondary study with the purpose of identifying, selecting, analyzing and interpreting all existing data related to a specific subject area, research question or interest phenomenon. The design of the systematic review reported in this article began in March, 2020. Fig. 4 (PRISMA) depicts the research method utilized in this study for systematic literature review. This could only be possible by identifying research questions relative to the research objectives.

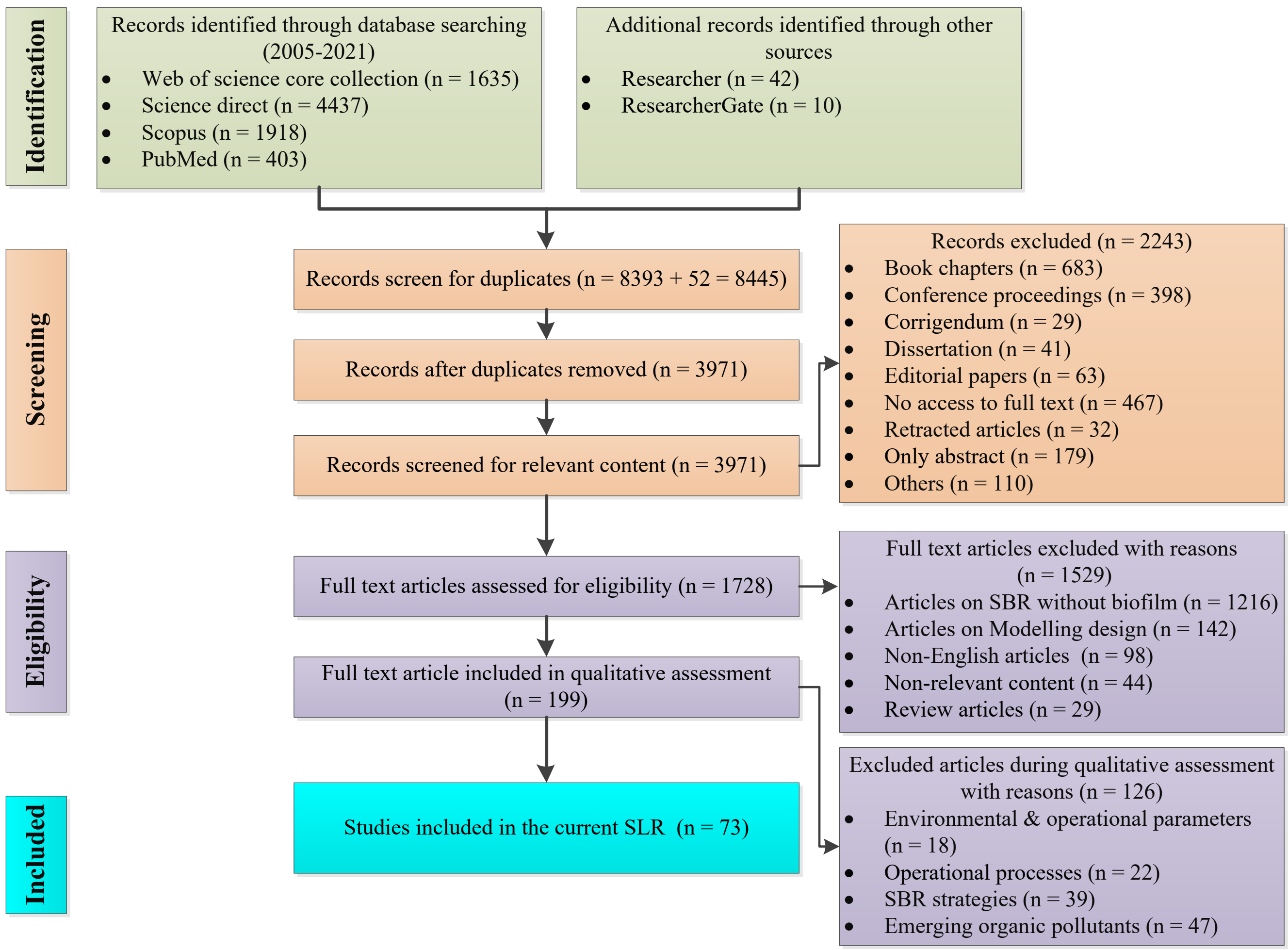


Fig. 4. PRISMA flow chart for the review process

3.1 Research questions

With the expanded utilization of SBBR for waste management resulting to gas generation and pollutant removal in wastewater, it has become necessary to study the function of biocarriers in the biological system. Therefore, the primary aim of this study is to form a perception of biocarrier materials performance in SBBR systems. The study further highlights how SBBR systems using different biocarrier materials become more significant than other biological treatment methods.

In a typical systematic literature review (SLR), the design of review process is often guided by research questions. Therefore, specifying the questions and developing strategy to answer them is the most important part of any SLR. In this present SLR, the main objective was to answer the questions regarding the performance of biocarrier materials in SBBR systems. Hence, the specific research objectives and the corresponding questions developed for this study as stated in Table 1 below are:

Table 1. SLR objectives and questions

	Objectives	Questions
1	To identify the most common biocarriers applicable in SBBR system and their properties	What are the distinguishing properties between the most common biocarriers used in SBBR?
2	To verify the performance of biocarriers in SBBR during nutrients removal and biofilm formation	How does biocarriers and their properties affect SBBR performance during nutrients removal and biofilm formation?

3.2 Search strategy and criteria

The search process adopted in this review focused on four (4) electronic databases (see Fig. 4) to obtain publications related to the review scope. The databases were selected due to the fact that they are among the most inclusive and exhaustive scientific databases with wide data coverage, encouraging the production process of sound bibliometric investigations. Additional records were found using other sources as "Researcher" and "ResearchGate".

Pertaining to search criteria, it is worthy to note that, the coverage of this review was from 2005 to September 2020, including the following studies, given that several materials are still being investigated for potential application as biocarriers (e.g waste materials). The collection of data was completed on the 1st September, 2020.

To obtain biocarrier investigations over the scientific community, the next stage of the literature search was to insert two phases in the document search: TITLE-ABS-KEY "Sequencing batch reactor" AND "Biofilm". The word "biocarrier" was not used because most authors do not use the word in their article tittles. Instead, they use "biofilm" since the formation of biofilm is their major target. Finally, in order to obtain specific articles across the scientific community that focused on biofilm formation, which is practically impossible in an SBR system without a biocarrier, authors agreed and adopted the search by "Tittle". Remarkably, we did not find any SLR article that investigated the impacts of different biocarriers in SBR for wastewater treatment.

3.3 Screening

The screening stage in this study began with duplicates removal. Several duplicates were found because four (4) electronic databases were considered plus other additional two (2) source. Records left after duplicates removal were further screened for relevant content. At this point, the following categories were removed: book chapters, conference proceedings, corrigendum, dissertation, editorial papers, no access to full text, retracted articles, only abstract and others.

3.4 Inclusion and exclusion criteria

The final search was conducted to obtain the most important articles for utilization in the SLR described in this review. Clear contributions, valid research and data were the basis for article selection upon which the study was carried out. The inclusion and exclusion process were intended to select the appropriate studies, extract and synthesize the data needed [34]. In the initial stage, a large number of articles were collected from the chosen databases for analysis. The bias for the articles inclusion or exclusion threshold was carried according to Eq. (4) defined in terms of included articles relevance ratio (R) for a given year (y), the number of matched keywords (k), the total number of proposed keywords (n) and the number of initial papers in a given year (P) according to equation (5).

$$R^y = \frac{\sum_i^n \frac{k_i}{n}}{P^y} \tag{4}$$

Included and excluded articles used in this research were supported by equation (5) [35, 36].

$$f(k_i) = \left\{ \begin{cases} included, & R^y < \frac{k_i}{P^y} \\ excluded, & (otherwise) \end{cases} \right\} \tag{5}$$

The inclusion guidelines for articles chosen after filtering for the full review due to their quality and well-known relevance are detailed in Fig. 4.

3.4 Taxonomy

Fig. 5 depicts the taxonomy built on the basis of the literature trends; it also represents and differentiates between the various subcategories in the main category in order to prevent overlaps . Within the restrictions of this review, the taxonomy highlights the biocarrier materials used in SBBR systems on the basis of their significance, which it considers to be the key concern in the field of biological wastewater treatment systems.

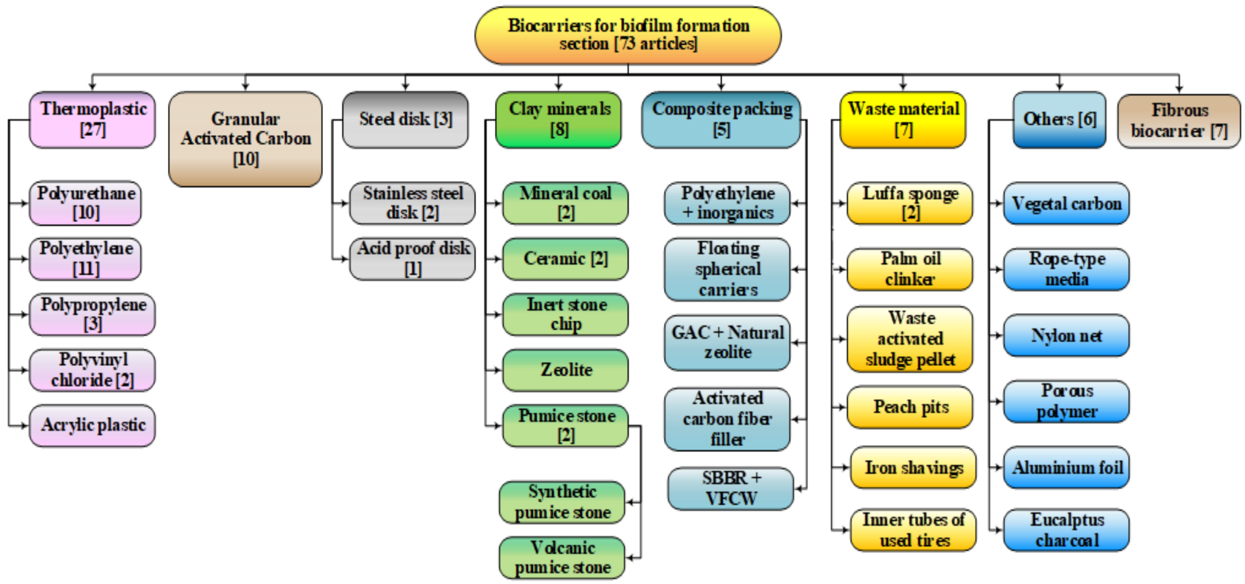


Fig. 5. Taxonomy of research literature on biocarrier materials

3.5 Descriptive data analysis

The scientific interest for the use of both natural and synthetic materials as biocarriers has over the year 2005-2020 maintained similar trend despite technological advancement. But, one thing that would interest you is that, during this period, a wide range of materials have been explored. This was possible as these materials are been subjected to characterization which displays the material property before and after use in SBBR. Fig 6 shows that studies on this specific topic in the year 2012 has eight (8) as the highest number of articles followed by years 2015 and 2019 that both have seven (7) articles. It could also be observed that year 2010 with a single article has the least number of articles.

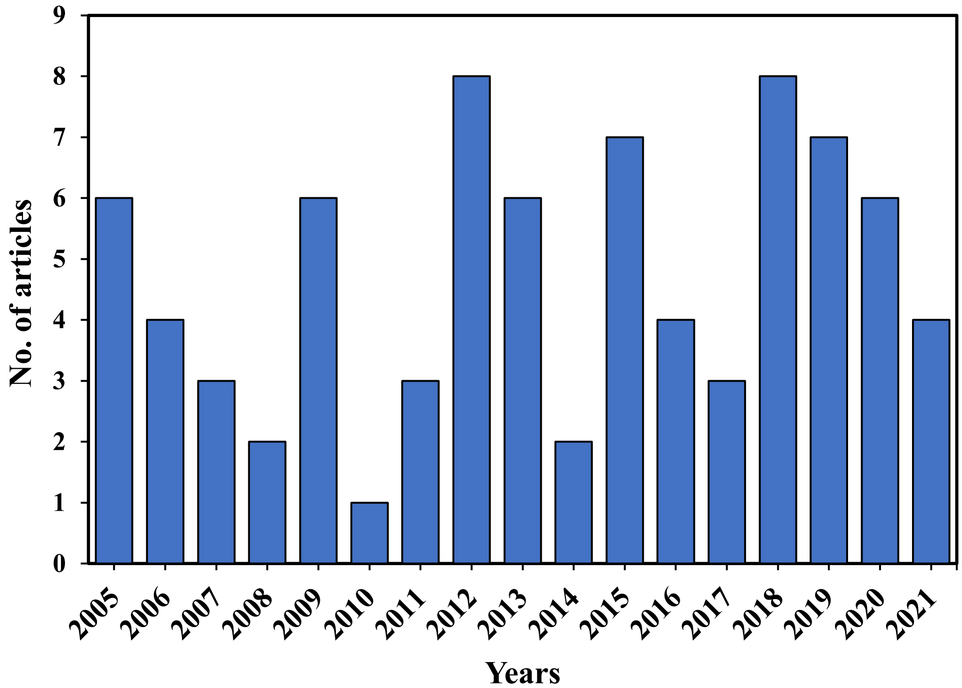


Fig. 6. Evolution of published article on biocarriers per year

A total of forty-two (42) different sources of publications were considered in this review. This reveals the fact that learners and publishers are beginning to proffer significance to the application of different materials as biocarriers in SBBR systems due to their potential impact on biological treatments. Surprisingly, only five (5) sources published more than two articles which amounts to a total of twenty-six (26) articles. Of the five (5) sources, the most prolific journal with 10 papers is "Bioresource Technology" which main goal is the publishing research in biological wastewater treatment. The second highest is the "International Biodeterioration & Biodegradation" and "Journal of Environmental Management" with each having five (5) articles. It could also be observed from Fig. 7 that, the journals "Environmental Technology" and "Water Science & Technology" both had three (3) articles.

About Ten (10) journals covering 13.7% of the total seventy-three (73) articles considered in this review had two (2) articles each. The journals are: Advanced Materials Research, Desalination and Water Treatment, Ecological Engineering, Journal of Environmental Sciences, Journal of Ecological Engineering, Journal of Environmental Engineering, Journal of Hazardous Materials, Korean Journal of Chemical Engineering, Water Research, Water Air Soil Pollution.

A total of twenty-seven (27) journals each have one (1) as the least number of published articles on biocarriers that met the inclusion criteria of this review. They are: Applied Biochemistry and Biotechnology, Asian Journal of Chemistry, Biochemical Engineering Journal, Biotechnology and Bioengineering, Brazilian Archives of Biology and Technoology, Brazilian Journal of Chemical Engineering, Asia-Pacific Journal of Chemical Engineering, Chemical Engineering Journal, Chemical Engineering Science, Chemosphere, Chinese Journal of Chemical Engineering, Desalination, Dyes and Pigments, Environment Protection Engineering, Environmental Progress & Sustainable Energy, Environmental Science & Technology, Frontiers of Environmental Science & Engineering, International Journal of Chemical Reactor Engineering, International Journal of Environmental Research, Journal of Bioscience and Bioengineering, Journal of Environmental Biology, Journal of Environmental Science and Health, Journal of Industrial and Engineering Chemistry, Journal of Water Process Engineering, Jurnal Teknologi, Process Biochemistry, Science of the Total Environment.

It is vital to note that, when assessing these sources, the majority have a very sectoral reach for engineering application of biological wastewater treatment. For this reason, journals with a strong environmental scope, as the "Journal of Environmental Management", "Bioresource Technology" and "International Biodeterioration & Biodegradation" carved a widening niche in biocarrier studies.

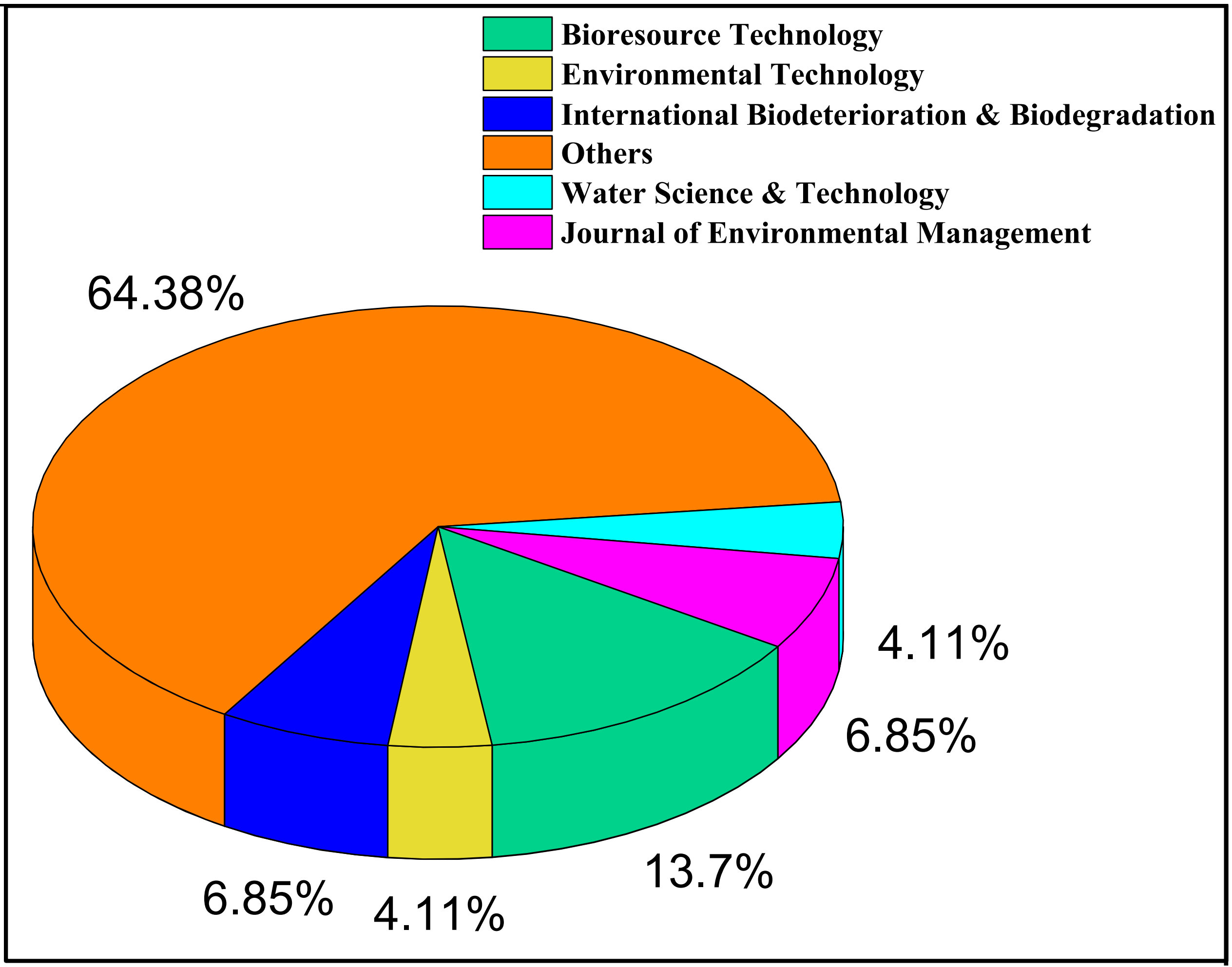


Fig. 7. Percentages of articles published per journal

Content analysis was utilized to know the geographical locus and sectoral focus of the articles. Although, both were not written in the keywords, the content analysis was conducted to make categorization of the articles in terms of location. It is interesting to observe that different countries are involved in biocarrier material study. Nineteen countries across the globe were found. China, Malaysia and Brazil with twenty-three (23), thirteen (13), and eight (8) articles respectively are the three major countries with the highest published articles considered in this study (see Fig. 8). India, Japan, Taiwan and Thailand published two (2) articles each, Poland Iran published four (4) and five (5) articles respectively. Least of all were countries that published only one article each. They are: Canada, Korea, Mexico, Portugal, Singapore, Spain and Turkey.

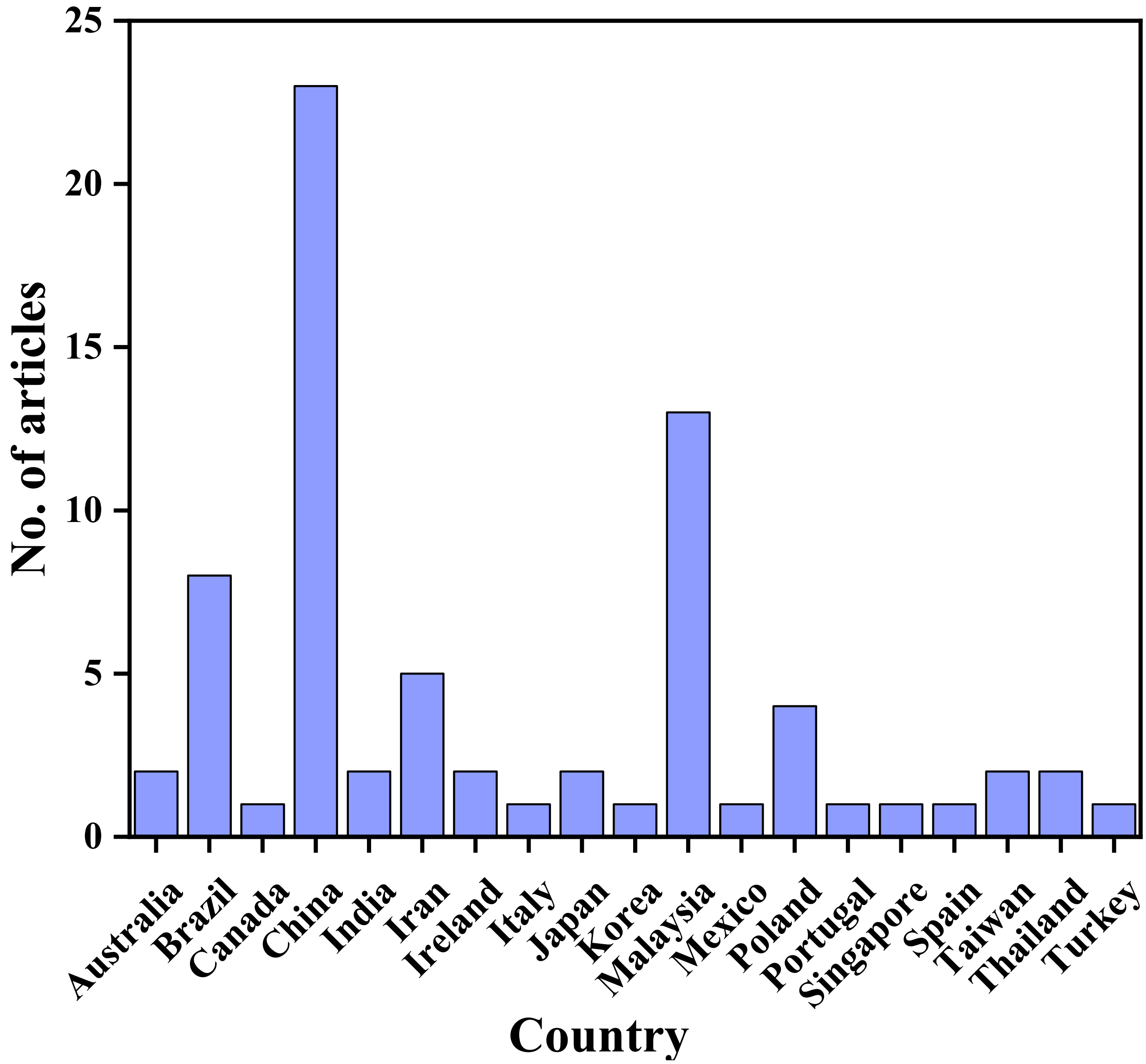


Fig. 8. Biocarrier research studies versus countries

4.0 Biocarrier properties

Biocarrier material selection is critical to wastewater treatment efficiency. The external and internal layers of the biocarrier are typically dominated by aerobic and anaerobic microbial communities. The total sum of the aerobic and anaerobic layers are used to measure the total biofilm thickness [19]. Several biocarrier elements such as polypropylene (PP), polystyrene, activated carbon, polyethylene (PE), polyurethane (PU) has been used to increase the SSA available for bacterial growth and yield different results. Therefore, it is very important to study and understand the properties of different materials before adopting them as carrier elements in a bioreactor.

The present section deals with the properties of several natural and synthetic biocarriers suitable to be used for biofilm formation in SBBR systems. For the sake of clarity, the comparison among properties of various biocarrier materials (thermoplastic materials, waste materials, minerals, steel disc, GAC) etc are reported and compared against each other in Table 2.

4.1Thermoplastic materials

4.1.1 Polyurethane (PU)

Polyurethanes are formed by reacting a polyol (an alcohol with more than two reactive hydroxyl groups per molecule) with a diisocyanate or a polymeric isocyanate in the presence of suitable catalysts and additives. Because a variety of diisocyanates and a wide range of polyols can be used to produce PU, a broad spectrum of materials can be produced to meet the needs of specific applications. The different PU types existing in literature are: flexible polyurethane foam, rigid polyurethane foam, coatings, adhesives, sealants and elastomers, thermoplastic polyurethane, reaction injection molding, binders, waterborne polyurethane dispersions [37]. The cubic shape of thermoplastic PU foam is the most common type used in SBBR systems. The PU foam with an excellent setting for immobilization of biomass belongs to the support media type of Linpor. It is marked by great mechanical strength, high porosity, large-specific pore size, high absorption, corrosion resistant properties and low cost [38]. Combined anoxic, anaerobic and aerobic environment can be created by PU biocarriers. Biofilm protection from collision and fluid shearing by the formation of attached-growth biomass, carbon storage, and provision of sheltered anchoring points could be possible by the porous nature of the PU structure [39]. Apart from biofilm protection, other merits of PU biocarriers are: quick response to cycle time, short start-up period, changes in volatile solids loading rate and process stability. However, the porous nature of the biocarriers could lead to low mass transfer efficiency [40]. An SBBR stocked with PU biocarriers has been confirmed to be an adequate and inhibition-tolerant system for nitrification and denitrification process that may occur outer and inner layers respectively [41].

PU foam mostly described as a sponge media (see Fig. 9) can be available in different forms. These are: square, rectangular, circular, spherical, cubic etc. they are mostly described as sponge media. To immobilize biomass in a SBBR system, PU are used as support materials. They are mixed and compressed with a large quantity of sludge. After keeping in contact for at least 2 hours, the foam matrices could be transferred to a sieve, in which excess sludge are removed by washing the foam on the screen. The inoculated foam cubes are finally placed into the carrier holder material allocated inside the reactor encircling the agitator axle to appropriately capture the micro-organisms [42], until its complete filling. The PU common biocarrier holder materials include: glass baskets, perforated stainless steel basket (cylindrical), fish wire fixed into a plastic rack etc [33, 43]. In addition, colonization of PU foam cubes with anaerobic biomass appeared to be supported by the efficient physical retention of influent SS [44].

Surface morphology is very essential for PU foam characterization. Therefore, it can be analyzed by a scanning electron microscope (SEM). The outcome of the SEM does assist in assessing the PU foam cube 's total internal and external surface areas. PU foam pores are commonly considered to be spherical in appearance with a consistent diameter and hemispherical pores covering its external surface.

Using the assumptions that PU pores are spherical with hemispherical exposed pores. Each pore surface area (m^2) and volume (m^3) can be expressed by Eqs. (6) and (7), respectively:

$$S_i = \pi d^2 \tag{6}$$

$$V_i = \frac{\pi d^3}{6} \tag{7}$$

where d (m) is the average diameter of the pore. The total surface area can be calculated by dividing Eq. (6) over (7) to obtain the surface to volume ratio. Rearranging:

$$S = \frac{6V}{d} \tag{8}$$

where V (m^3) is the total pore volume of a cube of known weight and S (m^2) is the total surface area of a PU foam cube.

To obtain the value of V, foam cubes are being soaked in water to saturation followed by measuring the quantity of water infused into the pores. By dividing the overall surface area by the known weight of the PU foam cube, the total surface area per unit weight (m^2/kg) can be determined [39].

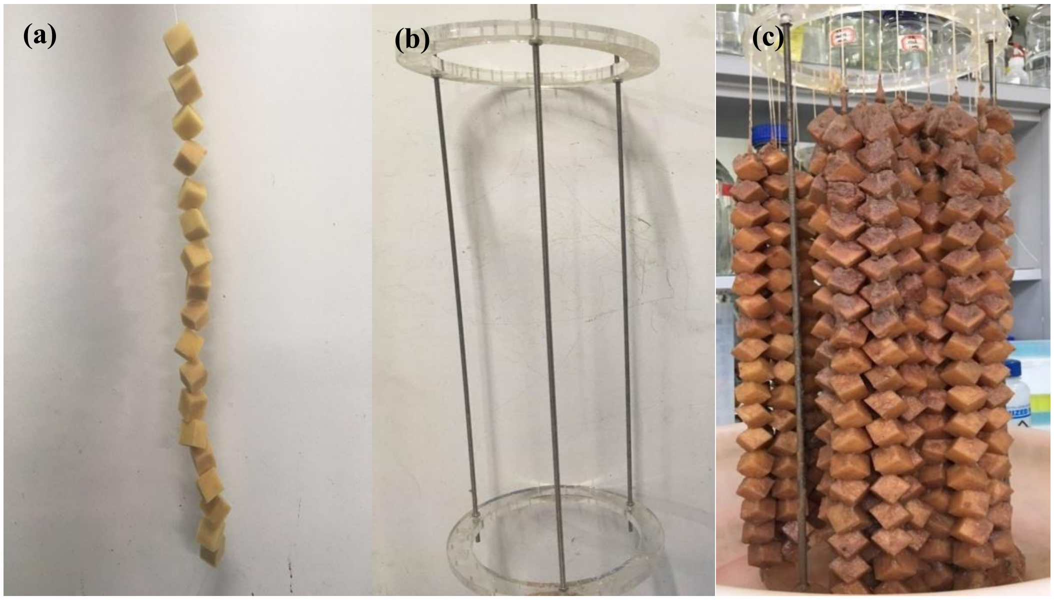


Fig. 9. Structure of immobile carrier used in the experiment: (a) carriers which strung together by using fish wire; (b) a plastic rack; (c) real photo of the immobile carriers (Adapted from [33]).

4.1.2 Polyethylene (PE)

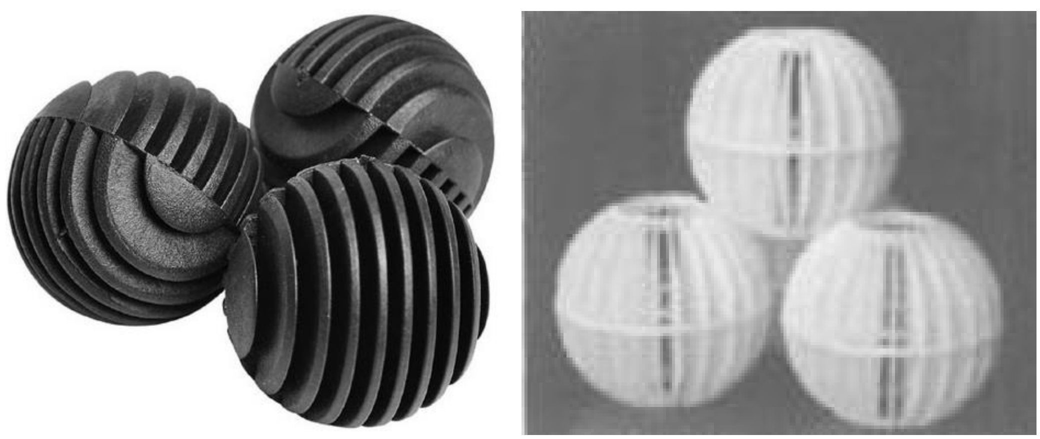
PE is a lightweight, variable crystalline structure and most commonly manufactured and used durable thermoplastic today. It is a man-made, homo-polymer, linear material mainly used for packaging [31]. It is also applicable to laminates, films, biocarriers, tubes, plastic parts etc. The biocarrier elements made of PE can be shaped like small cylinders with longitudinal fins on the surface and a cross in the inner layer of the cylinder [45]. Suitable and better SSA for biofilm growth could be provided by the inner layer of the cylinder [18]. For these processes, the cumulative SSA is considerably greater than the efficient SSA. Therefore, the most significant parameter that defines the performance of SBBR is the effective SSA [27]. The PE biocarrier density is less than water. This permits biocarriers to circulate and suspend within the reactor [26]. In order to get more lighting and optical energy for bacterial growth and enrichment, biocarriers can therefore rise to the surface during the non-aeration time [4]. Usually maintained in suspension with an airflow rate, PE biocarriers act as biofilm formation and microbe adhesion vectors [46].

The numerous PE biocarriers found in literature are Kaldnes media, PE beads, commercial ring-lace kinds of modified PE materials. The pumps and timing of operating cycles must be controlled by a time cycle controller device using the PE beads fixed by a PE net. Kaldnes media as illustrated in Fig. 10 is one of the most commonly used products as a biocarrier because of its simplicity in operation, high resistance, good total SSA and economic gain [17]. The Kaldnes media made of high-density PE (HDPE) exist as K_1, K_2, K_3, K_4, and K_5 [3, 47]. According to literature, biofilm media microscopy from several full and pilot scale moving bed biofilm plants showed that there was no evidence of biofilm germination on the surface of the smooth plastic elements using various Kaldnes PE media. Erosion incurred by the regular collisions between the pieces could be assumed as the explanation for this. Accordingly, internal surface of the plastic element can be used to compute the biofilm surface area [48, 49].

Fig. 10. Kaldnes biofilm carrier (Adapted from [17])

4.1.3 Polypropylene (PP)

PP is a thermoplastic polymer manufactured through chain-growth polymerization from the monomer propylene with wide variety of applications. It is partially crystalline and non-polar belonging to polyolefins groups. Multifaceted hollow balls, PP packing ring, membrane module containing hydrophobic PP dense hollow fibers, PP BioBall® carriers (see Fig 11a) etc have been used by researchers as carriers for bacterial immobilization [50]. BioBall® as a biocarrier made of PP has a spherical design is utilized by aquarists as mechanical and biological filter mediums [30]. The PP packing ring (see Fig 11b) are cylindrical in shape with a unique arrangement of three-dimensional hollow that could shield the biofilm from fluid shearing and create DO concentration gradients inside the biocarrier, presenting typical circumstances for SND. The PP rings are used to structure the PU foam, thus resulting to good bed porosity. It also has decussation for structural support at the center. Similar to some biocarrier materials, the shape of PP packing rings was regular, with large SSA. A study reported that packing rings shifted during aeration [21, 49].



(a) (b)

Fig. 11. Polypropylene (a) BioBall® carriers and (b) white suspended packing rings (Adapted from [21] and [30])

4.1.4 Polyvinyl Chloride (PVC)

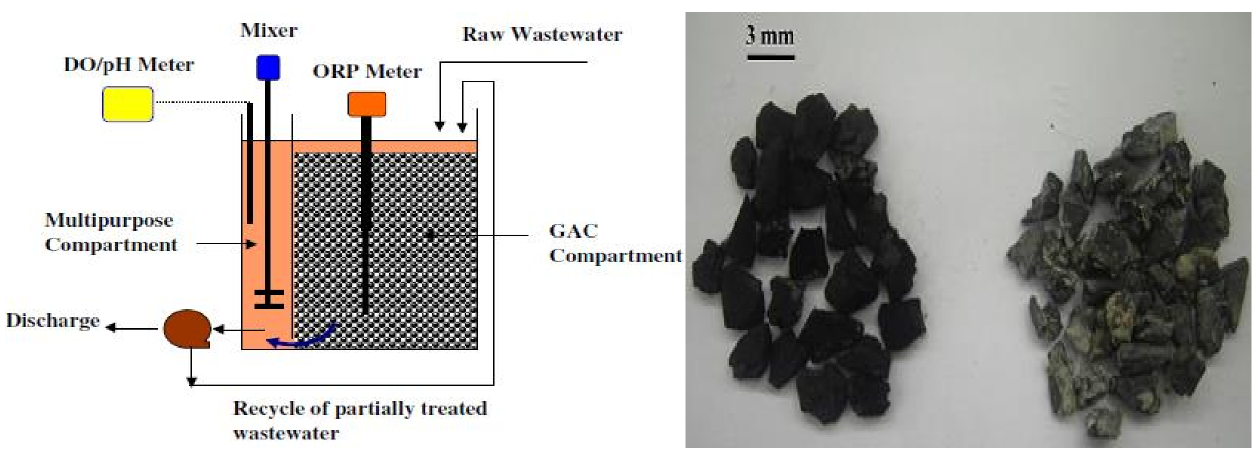
PVC is the third most commonly manufactured and high-strength thermoplastic material on earth, which is essentially rigid and flexible in shape. Available in granular or powder form, it is a brittle, white, solid substance. It is being used in a range of applications in the health care, building and construction, automotive, electronics and other industries, in products ranging from blood bags and tubing, piping and siding to windshield system parts, biofilm formation, wire and cable insulation and more, because of its versatile properties such as: low cost, lightweight, simple processability and durability. To achieve an adequate surface area, PVC plastic modules can be assembled manually in a reactor with a stainless-steel frame attached to a pneumatic piston meter placed above the bottom of the reactor to accommodate these modules. This is to provide space for the lower level switches to work freely and isolate the modules' bases from any sludge build-up [51]. Pneumatic piston, timers, and limit switches are used to vertically move the modules in and out of the wastewater in cycles [25].

4.1.5 Porous polymers

Porous polymers possess large SSA, high sphericity and structural strength, and a density similar to that of water. The biocarriers are easy to fluidize but not easy to split, and thus ideal for the immobilization of microorganisms. Circular fluidization of biocarriers in SBBR system has numerous benefits, including: (i) the breaking down of air bubbles that improves the efficiency of mass transfer and enhancing interaction between biofilm and contaminants; (ii) reinforce the collisions between biocarriers to renew biofilm immobilization on time and consequently foster biofilm activity; (iii) decreased short circuiting, clogging, and excessive pressure loss occurrences that frequently occur in moving biofilm or fixed bed SBBRs [52].

4.2 Granular Activated Carbon (GAC)

The filtration by GAC typically used in wastewater treatment was initially intended to eliminate organic matter by adsorption. However, once the active sites are depleted, the GAC will no longer be useful. Therefore, the spent GAC is either dumped or revived as secondary waste. GAC can be re-generated from apricot shells, coconut shells, coconut husks, bamboo, walnut shells etc [53]. Most of these are commercially readily available [54]. Reports have it that, the immobilization of microflora as biofilm on GAC particles (see Fig. 12a and 12b) results in high biomass holding, allowing the process to significantly operate at higher liquid throughputs and OLR [55]. This is because of the numerous advantages possessed by GAC that result in high process stability and increased substrate removal efficiency: high surface area, porosity, large adsorption capacity, rough medium and continuous GAC bio-regeneration [56, 57]. Since GAC's low specific gravity enables fluidization even at less upflow velocity, its capacity for sorption shields the attached biofilm from shock loads [58, 59]. As an adsorptive biocarrier medium, GAC serves as a buffer to decrease toxic chemicals level during process activity [60, 61]. The adsorption capacity of spent GAC may be restored by chemical and thermal recovery on spent GAC to act as a support media for microorganisms' attachment and growth. However, these methods have significant disadvantages, such as spent GAC disposal issues, high energy consumption, carbon loss, loss of adsorption capacity and high cost. In addition, the spent GAC surface could be regenerated for further adsorption by microorganisms that grow on it. Therefore, the use of spent GAC as a support medium for biofilm formation could significantly contribute to minimizing waste generation and as well adding value to the material in question [62]. The method is cheap, simple and ecofriendly. On the contrary, organic matter saturation is an unavoidable limitation of GAC adsorption process. An undrained organic matter GAC is considered poor since there is a short service life of around 6-12 months for GAC. Consequently, the biofilm can recover GAC after a period of net adsorption to maximize its lifetime.



(a) (b)

Fig. 12. (a) Schematic diagram of GAC-biofilm configured SBCR and (b) image of biofilm GAC acquired from pilot-scale GAC-SBBR along with virgin GAC prior to its addition to the reactor (Adapted from [60] and [55]).

4.3 Waste materials

4.3.1 Waste activated sludge (WAS)

Several materials have been utilized as biocarriers for biofilm formation. Most of them were, however, solemnly used to immobilize anammox bacteria, whereas AOB and denitrifiers are also present in the SNAD and CANON system [58]. Therefore, new biocarriers that are highly effective in biomass retention within the reactor and are capable of starting the SNAD process with long-term operational stability in a very short period are required. Biomass carriers prepared by using WAS, chemical additive vesicant and red soil (see Fig. 13a, b and c) have been documented to be appropriate. Red soil and chemical additive-vesicant added to the mix are responsible for providing stickiness and hardness to the biocarrier respectively [29].

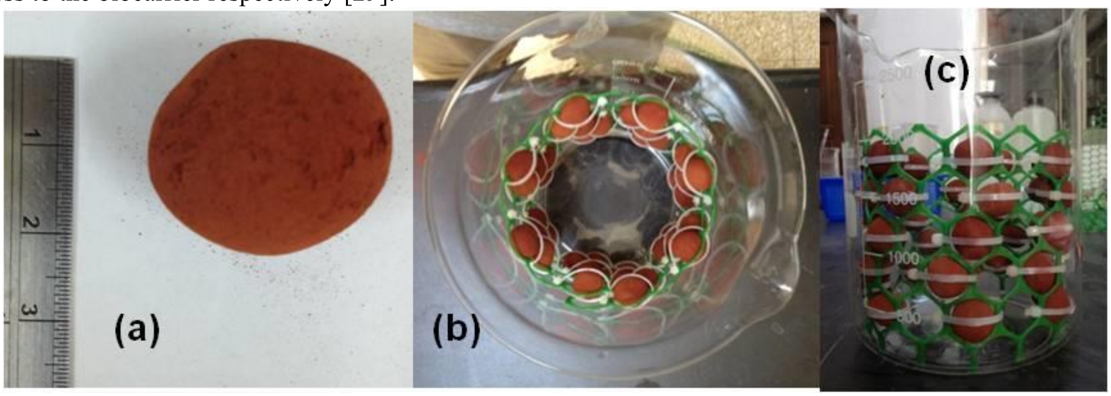


Fig. 13. Images of biomass carrier showing diameter (a), and arrangement in the reactor: top view (b), side view (c) (Adapted from [29]).

4.3.2 Inner tube of tires

A biocarrier sourced from the inner tube of used tires in the form of cubes can be applied in an SBR system to increase the generated bio-sludge quality and system efficiency. This is feasible as the material possess properties such as: reusability without any regeneration, non-biodegradability and good sedimentation. Unlike biocarriers made from thermoplastic materials, the density of carrier from used tires is greater than the density of water. This attributes to the good settling characteristic of the material [63].

4.3.3 Palm Oil Clinker (POC)

POC is a by-product of the incineration process resulting from the exhaust stream of the palm oil industry plant in a steam boiler producing electricity. It is a black solid waste material seen in Fig. 14, which causes environmental sustainability problems. POC has an irregular shape, rough surface that contain huge number of voids and pores. Its lightweight nature is attributed to the extremely porous nature of its interior portions. With a chemical mixture of Al_2O_3, Fe_2O_3, and SiO_2, POC is regarded a firm and non-biodegradable substance. Because of its easy operational capability, affordability and the pace at which it improves the contact surface area for biomass aggregate, POC is considered acceptable for biofilm formation in SBBR [64].

Fig. 14. Chunks of POC (Adapted from [64])

4.3.4 Peach pits

Thermoplastic materials have been widely studied for utilization in SBR serving as biocarriers for biofilm formation during wastewater treatment. However, they are neither supportive to the community nor cost-effective. In terms of environmental issues, the use of carbonaceous eco-friendly and wood-based biocarriers for proper immobilization of bacterial consortia as biofilm may be a promising choice. Peach pits depicted in Fig. 15 could be used as sustainable biocarriers due to their high porosity, firm structure, cheapness, and abundance. Pits must be removed from the reactor and drained for 1 h at 105°C to compute the mass of biomass attached to the peach pit surface. The dried biocarriers will be weighed to calculate their total mass (M_t) with biofilm. Afterwards, pits will be rinsed and sanitized to assess the weight of biofilm-free (M_c) carriers [9]. Lastly, the volume of biofilm fastened over the surface of 20 peach pits (BS_{20}) can be calculated according to the following equation:

$$BS_{20} = M_t - M_c \tag{9}$$

$$\mathrm{BS} = BS_{20} \text{ x } \frac{585\, L^{-1}}{20} \text{ x FR} \tag{10}$$

When the filling ratio (FR) is 100%, 585 carriers can flow into the unit volume of the reactor. The 585 used in equation 10 above is the total number of carriers required in unit volume of a reactor. It depends on the average volume of each carrier to be used [9]. Consequently, employing Eq. 10, it is possible to measure the total quantity of biofilm in the reactor (BS) [65].

Fig. 15. Typical peach pits

4.3.5 Luffa Sponge (LS)

The LS is usually organic and biodegradable, naturally soft, yet durable materials. It is an annual vine that is edible when young, and widely used as a bath sponge, with large cylindrical fruits and tendrils. However, young fruits that are < 7 inches long and green can be consumed as fresh cucumber or squash replacement. The fruits fibrous interiors of the LS gourd plant (*Luffa aegyptiaca Mill*) shown in Fig. 16 are the portion used as biocarrier. As a natural resource, it begets no harm or ill effects on microorganisms. LS facilitates denitrification by serving as a carbon source. In fact, it is an adequate habitat for microbes and easy to enrich the biofilm of microorganisms [66]. According to literature, LS is ideal for use in immobilization processes due to the fact that: (i) the natural structure of its micro-fiber offers voids where metabolites and substances are exchanged (ii) it has a broad specific surface with less density than water and is also rich with hydrophilic surface cellulose and lignin [67].

Fig. l6. Structure of luffa cylindrical sponge (Adapted from [67]).

4.3.6 Iron shavings

Iron shavings can be utilized as biocarrier materials. They are commonly produced from mechanical cutting of cast iron ingots with a reduced iron content and SSA than nano-iron particles or iron powder purchased commercially. Because of their comparatively inferior surface area-to-weight ratio, they are ideal for promoting iron-assisted autotrophic denitrification. Iron shavings can be purchased from a machinery factory [68].

4.4 Steel disk

A biocarrier consisting of a bundle of eight fully submerged stainless-steel discs, co-axially joined and rotated by an electric motor in a bioreactor shown in Fig. 17 is said to allow biomass preservation in the form of biofilm coating on the discs [69]. It also conducts wastewater stirring and periodical aeration, discharges the entire amount of wastewater and enables the sloughing of surplus biomass. The disks submersion rate is to 100 % [32, 70].

4.5 Clay minerals

4.5.1 Zeolite

Zeolites are aluminosilicate microporous minerals widely used for concentrated NH_4^+-N wastewater treatment as industrial catalysts and adsorbents due to their outstanding selectivity for many cations (see Fig. 18). Due to its superior adsorption characteristic, molecule sieve feature and ion exchange, natural zeolite is one of the most desirable materials for biological and physical wastewater treatment. It has a good selective ability to adsorb NH_4^+-N and has been shown to support both aerobic and anaerobic microorganisms [71]. In order to provide a healthy living environment for AOB development, it interestingly serves as an excellent biocarrier suitable for AOB adhesion and enrichment. NH_4^+-N concentration in zeolite-filled SBBR can achieve a dynamic equilibrium of NH_4^+-N through ion exchange, which may also provide sufficient free ammonia levels in the reactor to maintain nitritation. It is assumed that a suitable FA range could be given and excellent partial nitrification achieved by maintaining a desirable NH_4^+-N concentration for Nitrite Oxidizing Bacteria (NOB) inhibition [72].

4.5.2 Inert stone chips

Using an inert stone in an SBR, fixed bed has to be placed up to certain height level with a reasonable void ratio after immobilization of anaerobic mixed consortia. These packing material can be used to encourage the germination of H_2 producing acidogenic mixed microflora [20].

Fig. 17. SBBR disc media (Adapted from [32, 69, 70]).

Fig. 18. Zeolite

4.5.3 Mineral coal (MC)

MC is a hard-combustible sedimentary rock of plant origin, created by natural processes with some quantity of mineral impurities, that appears in the form of beds or layers among other sedimentary rock (see Fig. 19). It is a complex rock consisting of many natural organic and inorganic components, found in underground beds with different physicochemical, technological, and mechanical properties. It is often regarded as a natural composite. By removing the need for an internal system to maintain the bed particles, the replacement of many biocarriers with MC as a denser material will simplify the reactor design. MC is said to be cheaper than several other biocarriers [73].

Fig. 19. Schematic representation of a typical MC (Adapted from [73]).

4.5.6 Ceramics

Ceramics can be utilized as vectors for biofilm formation and microbe adhesion due to the porous nature of its surface structure. Since its density is slightly greater than water, the biocarrier could fix well on the supporting sheet. In order to obtain adequate nutrients for growth and reproduction during the aeration cycle, bacteria and algae on the biofilm could have detailed interaction with wastewater. And during the time of non-aeration, the two phases could be completely separated. As biocarriers remain on the supporting layer to obtain optical energy and more illumination that is beneficial for algae growth, AS can easily settle down to the bottom of the reactor [74].

4.6 Fibrous carriers

The fibrous packing media shown in Fig. 20 can shield the biofilm and provide bacteria with an ideal environment to flourish. It combines the features of semi-soft and soft biocarriers. The biocarrier offers a robust framework to protect bacterial cultures from the impact of operating excursions [14]. Fiber threads are normally filled into holes and connected to the reactor surface by various materials, such as iron wire, thereby creating a biofilm carrier [75]. In order to shape a huge surface area, filaments are spread and flexible carbon fiber yarns swung under the aeration condition with the water in the reactor so that the mass transfer in the biofilm could be enhanced [76]. In both the aeration and reverse flow region, these biocarriers can be packaged to shield the biofilm from fluid shearing and clash and to absorb enough microorganisms on the biocarrier. Fiber threads have high adhesion efficiency, large SSA, large biomass and evenly dispersed filaments around the block that can efficiently cut gas and increase the oxygen utilization rate, thus providing ideal conditions for SNAD [1, 10, 77]. The powerful adsorption and growth of organisms results from the rough surfaces of the fiber threads. The fiber threads could also be used as filter media to reduce effluents SS concentration, which subsequently decreases bioreactor volume and settling time. The utilization of the flexible fiber as biocarrier offers a high surface area for greater microorganism attachment resulting to increased biomass concentration [78].

5.0 Influence of biocarrier on biofilm formation, nutrients and organic matter removal

The literature study reveals in general that several materials: thermoplastic (PU, PE, PP, polyester, polyvinyl chloride polyacrylonitrile), steel disk, minerals (coal, inert stone chips, ceramic), fibrous materials, GAC, waste materials (WAS, inner tube of used tires, peach pits, iron shavings, palm oil clinker, luffa sponge) etc. are used in SBBRs to enhance the systems performance. The differences in their performance as depicted in Table 4 would be thoroughly discussed in this sub-section as it directly affects nutrients removal and biofilm formation.

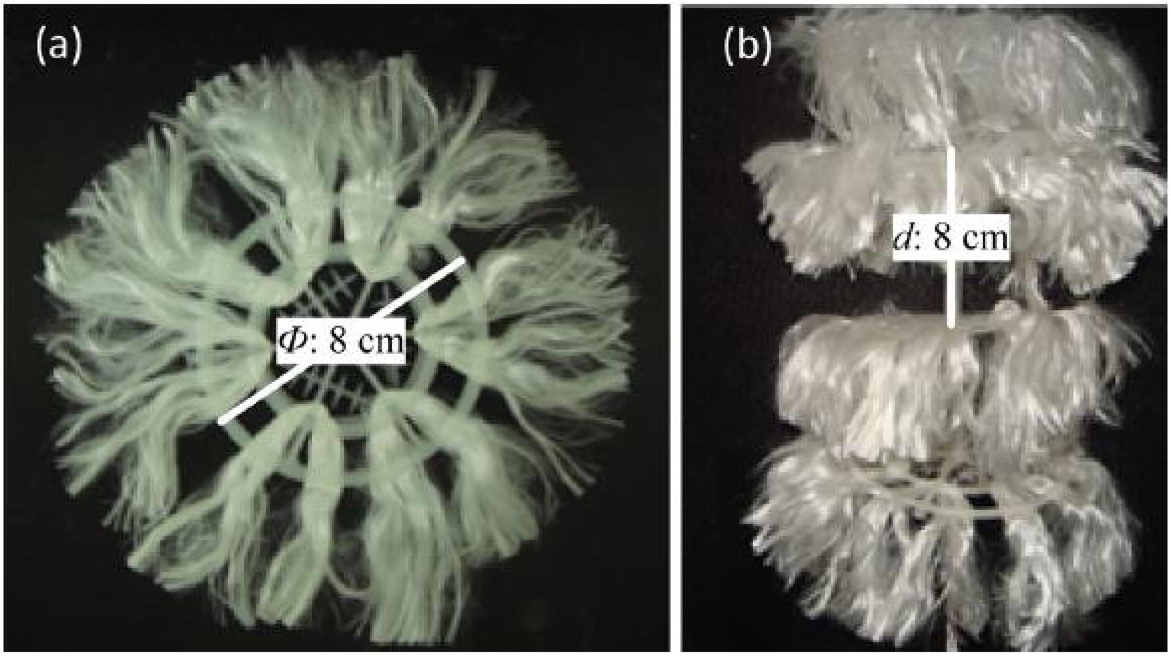


Fig. 20. Pictorial view of fibrous packing media used in SBBR (Adapted from [14]).

5.1 Biocarrier influence on nutrients and organic matter removal

5.1.1 Thermoplastic

5.1.1.1 Polyurethane (PU)

The improved performance usually experienced for SBBR systems is largely because of the appearance of attached-growth biomass [43, 44, 79, 80]. The application of Linpor plastic PU foam was explored and achieved complete nitrification even at higher organic loads because the average mean cellular retention times (MCRT) was 22.9 days when used as a biocarrier by Valdivia et al., [81]. The greater MCRT value played significant role towards achieving better nitrification by Linpor. Although denitrification was greater at lower organic loads, at higher organic loads > 3.0 $gCOD/m^2 \cdot d$, better removal of COD and NH_4^+-N could be attained. With values ranging from 15-20 mg/L, Linpor achieved the highest NOx-N yield. After the cycle operation, the nitrate concentration increased and ultimately decreased.

According to Sarti et al., [38] in their work, the ultimate quantities of COD removal observed were the direct product of methanogenic action. It may also be linked to the physical preservation of SS in the PU foam rather than to the formation of a proper biofilm by microbial degradation. The carbon source and the presence of anoxic zone were responsible for the promotion of SND processes alongside an improved TN removal in an MBSBR-PU biocarrier [66]. Throughout the operational duration, almost complete NH_4^+-N removal with a medium effluent concentration of 0.5 mg/L was achieved. This suggests that acclimated PU foam cubes did not perform any significant role in the process of assimilation [41]. The removal of NH_4^+-N may also be credited to the reality that the high pores of the PU biocarriers have been trapped by many microorganisms. However, in a study by [37], the NH_4^+-N removal efficiency has been reported to noticeably decrease as nitrogen loading rate (NLR) increases. Thus, this increase in NLR, NOB inhibition coupled with decrease in DO concentration during the aerobic phase were responsible for Nitrite-N accumulation.

In another study, it has been reported that for nearly 8 months, safe and highly effective nitrogen elimination (> 90 %) was attained. Consequently, Zhang et al., [33] stated that the application of porous sponge biocarriers could provide a strong benefit for biomass immobilization and boost the retention within the reactor of anammox bacteria.

Table 2. Physical properties of biocarriers

Biocarriers	Size	Shape	Density	Surface area	Porosity (%)	Others	Reference
PU	20 x 20 x 20 mm		0.022 g/cm^3	>20,000 m^2/m^3	>97	Pore size (25 ppi)	[40]
	50 mm W	Cubes	23 kg/m^3				[38]
	2 x 2 x 2; 3 x 3 x 3; 4 x 4 x 4 (cm)	Cubes	87.4 ± 5.3 kg/m^3			Average pore diameter (661 ± 53 µm); pore count (42 ± 7 per 25 mm); total surface (177 ± 3 m^2/kg)	[39, 41]
	375 cm^3	Cubes	1 g/cm^3				[33]
	1 cm L		23 kg/m^3		95		[43]
			0.3 – 0.5 g/cm^3	900 m^2/m^3	97	tensile strength, 120 kPa	[66]
	Ø 0.6 cm	Cubes	0.023 g/cm^3	43.8 m^2/g	92	mean pore diameter, 543 µm	[79, 82]
PE	Ø 17 mm; 10 mm H	hollow, star-shaped		407 m^2/m^3	74		[46]
	Ø 8 mm, 16 L; 7 mm H		0.95 g/cm^3	690 m^2/m^3		Dp, 2 mm (Dp = equivalent diameter)	[47]
	Ø 9.1 mm; 7.2 mm L		0.96 g/cm^3	150 m^2/m^3			[17]
	Ø 9 mm; 7 mm H	Cylindrical	145 kg/ m^3	800 m^2/m^3		Bioflow 9	[26]
	1.5 x 1.5 x 1 cm		0.95 g/cm^3	415 m^2/m^3		Weight 0.43 g	[45]
	Ø 19 mm; 14±2 mm H			369.6 m^2/m^3	23	Weight, 87±3 N/m^3	[3, 48]
	Ø 25 mm; 12 mm H	cylindrical	110 kg/ m^3	500 m^2/m^3			[18, 27]
	Ø 25 mm; 10 mm H		0.95 g/cm^3	460 m^2/m^3			[31]
	Ø 10 mm; 7 mm H		0.17 g/cm^3	500 m^2/m^3	44	bed height, 0.17-0.33 m	[81]
	Ø 0.5 cm	cylindrical	0.40 g/cm^3				[82]
	Ø 17 mm; 10 mm H			407 m^2/m^3		*DupUM*	[49]
	Ø 9.5 mm; 10 mm H			640 m^2/m^3		*Biolox10*	[49]
PP	1000 mm L	membrane module and a cylindrical plastic shell				thickness of hollow fiber membrane (30–40 µm); intra membrane pressures (0.10MPa and 0.15MPa);	[50]
	Ø 19.0 mm	spherical	0.92 g/cm^3	378.0 m^2/m^3		BioBall®, weight, 1.7 g	[30]

			0.95 g/cm^3				[67]
	Ø 32 mm; 30 mm H			320 m^2/m^3		*Bioflow30*	[49]
PVC	200 x 200 x 180 mm	Vertical columns		290 m^2/m^3		Biodeks PVC sheets (BIOdek, UK)	[25]
	0.29 x 0.21 x 0.26 m	Vertical columns		290 m^2/m^3		Biodeks media (Munters, UK)	[51]
Acrylic plastic	Ø 5 cm; 1.25 cm H	Cylindrical	0.96 g/cm^3	0.03 m^2		Weight, 2.40 g	[8]
GAC	~1.5 -2 mm		1250 kg/m^3			residue on ignition (600 ◦C): 5%; loss of drying (120 ◦C): 10%); Feed rate: 40 g/L	[53, 58]
			560 g/L			GAC exhibits limited service life of ~ 6–12 months.	[54]
	Ø 1.30 mm; 0.25-2.00 mm		1200 kg/m^3				[57]
	10-30 mm		0.85 g/cm^3	500-1000 m^2/g		specific gravity, 2 g/cm^3	[71]
Inner tube of used tires	2 x 2 x 2 mm^3	Cubes	1.925±0.21 g/cm^3	0.39±0.027 m^2		Volume of 8.0±0.74; weight, 0.0154±0.0022 g	[63]
Iron shavings				0.002634 m^2/g		Weight, 20 g;	[68]
Palm Oil Clinker	25- 35 mm		742-782 kg/m^3			Specific gravity: 1.64-1.80	[64]
LS		Cylindrical	0.77-0.78 g/cm^3				[67]
		Cylindrical	0.018-0.05 g/cm^3	850 m^2/m^3	85-95	Tensile strength, 11.1 MPa	[66]
Peach Pit			1.134± 0.064 g/cm^3			Weight, 7.025 ± 0.342 g; volume, 6.2 ± 0.258 cm^3	[9]
Steel disk	Ø 0.10 m			25 m^2/m^3		thickness 1x10^{-3} m of each disk, distance btw disk, 20 mm/2 cm; rotation speed, 60 rpm	[32, 69, 70]
Zeolite	Ø 80 - 200 mm			412.67 m^2/g		Adsorption capacity of NH_4^+-N, 10.41mg/L; Pore volume, 0.073-0.23 cm^3/g; Pore size, 2.25-3.82 nm	[72]
Inert stone chips	2.5 cm×1.5 cm				49		[20]

MC	Ø 40 – 80 mm				50	Weight, 500 kg	[73]
	Ø 20 – 40 mm				52	biocarrier occupied reactor volume 7.8 L	[19]
Ceramic	Ø 16 ± 3 mm	Spherical	1.1–1.2 g/cm^3	900 m^2/m^3	20.72–24.12		[74]
	Ø 4 – 8 mm	Sphere	1.4 g/cm^3	480 m^2/m^3	38	bed height, 0.16-0.33 m	[81]
Flexible fiber	Ø 0.07 mm; 75 mm L			2200 m^2/m^3	>90		[78, 83]
Fibrous slag	Ø 3 – 8 mm; 2-3 mm L			30 m^2/m^3			[84]
Plastic ball and GAC	Ø 3 cm, 2-3 mm		1111 kg/m^3			Plastic ball, total volume of 2.2 m^3 Volume occupied by the GAC 0.14 m^3	[55, 56]
Floating spherical carriers (PU with PE shell)	Ø 60 mm	Spherical PE shell	1.21-1.23 kg/m^3	2.15-2.21 m^2/g	85	water holding capacity, 452.38%,	[85]
Suspended carriers (carriers with a cross inside and longitudinal fins outside)	Ø 50 mm; 60 mm H	Spherical PE shell	0.96-0.98 kg/m^3	1.23-1.25 m^2/g		water holding capacity, 5.41%	[85]
WD-F10-4 BioM™ (composite of PE with inorganics)	Ø 10 mm; 10 mm L	Tube chip	0.96-0.98 kg/m^3	900 m^2/m^3		Thickness, 0.4 – 0.6 mm	[65]
Natural zeolite	10-30 mm		2.1 g/cm^3	500-1000 m^2/g	20-100	hardness, 3 N/mm^2	[71]
Rope-type media	220 mm L; 30-40 mm W				95		[86]
Nylon nets				4,140 m^2/m^3			[87]
Porous polymers	Ø 0.32 mm		1,320 kg/m^3	5,357 m^2/m^3		wet stacking density, 1,010 kg/m^3, pore volume of 0.301 ml/g	[52]
Aluminum foil	Ø 10 mm; 10 mm H			500 m^2/m^3	95	specific gravity, 960 kg/m^3;	[88]
Vegetal carbon	Ø 0.5 cm	cube	0.51 g/cm^3		43	mean pore diameter, 1.9 µm	[82]
Eucalyptus charcoal	Ø 0.5 cm	Irregular pellet	0.51 g/mL	3.51 m^2/g	43	mean pore diameter, 1.9 µm	[79]

638 **Note:** L = Length; B = Breadth; H = Height; W = Width

It has been stated that seed sludge is primarily responsible for the improved efficiencies for the removal of COD and nitrogen in MBSBR as it might easily penetrate and adsorb the porous layer of the PU type. Therefore, affecting the production of biofilms in the form of a thin and cream-colored bio-membrane filled with microorganisms [40].

Bioparticle size exerts a decisive effect on the output of an anaerobic process under different operational conditions. In to the findings by Pinho et al., [42], authors concluded that, total volatile solids (TVS) of the PU foam biocarrier are not altered by changes in the bioparticle size, showing that the kinetic results were not affected by the amount of biomass contained in the device. In a similar study, also varying bioparticle size, SND processes in the MBSBR system resulted in increased TN removal with decreasing PU foam cube sizes [39]. The comparatively lower TN removal attained when larger PU foam cubes were used could be due to the view that larger PU foam cubes were not completely connected by biomass. This is in line with the classic theory as earlier stated by [42]. The elimination of NO_2-N concentration at ≈ 90% was achieved using 8-mL PU foam cubes, followed by 80 and 70% for reactors with 27- and 64-mL PU foam cubes respectively. The fact that the existence of a greater volume of PU foam resulted in the lower quantity of suspended-growth biomass involved in the nitrification process can explain the decreasing values of NH_4^+-N removal reported with an increase in biocarrier concentrations.

Comparing the performance of PU foam with some other biocarriers, it offered the quickest start-up and produced steady effluent COD samples [82]. Considering the above-mentioned significant effects of PU foam in nutrients removal, it is clear that NH_4^+-N removal rate decreases with increase in NLR and biocarrier concentration. The review results also revealed that the SBBRs with PU as biocarrier system has good capacity to withstand pollution and hydraulic loading.

5.1.1.2 Polyethylene (PE)

PE media has been suggested as an efficient biocarrier for biodegradation of organic compounds [4, 49, 80, 82]. The concentration of COD and the period of settling may be the significant factors in denitrification [31, 45, 46]. The improved efficiency of the reactor biocarrier material in the elimination of aromatic compounds following anaerobic decolorization was due to the lower COD concentration reported in the fixed-bed sequencing batch biofilm reactor (FB-SBBR) effluent [16]. Using PE, biofilm biomass decreases as the C/N ratio decreases [26]. With inadequate microbial denitrification activity at a low C/N ratio in the reactor, nitrate concentration remained high in addition to suspended growth due to attached growth on biofilm carriers [17]. The findings showed that at a low C/N ration, biofilm was a more suitable environment for nitrifying bacteria than activated sludge.

In an anoxic biofilm denitrification system, highly efficient nitrogen removal was achieved [27]. Due to the endowment of denitrifying bacteria on biofilm, nitrite reduction was also observed. NO_2^--N concentration was found to gradually decrease to 1.78 mg/L after one month, indicating that the capacity to lower NO_3^-–N to NO_2^--N was strengthened by biofilm growth and denitrifying microorganism enrichment on the biocarriers. In a Partial Nitrification-SBBR (PN-SBBR) cycle, biofilm system was highly adaptable to an unstable influent substrate concentration with high TN removal [18] and as the principal nitrogen species in the effluents after the creation of biofilm, NOB activity was completely inhibited with nitrite. NH_4^+-N concentration was rapidly reduced during stable activity because of the dilution process following influent augmentation, and modified during the anoxic denitrification step. Biosorption processes can be related to the decrease in NH_4^+-N concentration.

Study summarized that ammonium removal efficacy of SBBR was more eminent than that of SBR as the biocarrier made it easy to remove more ammonium from high strength ammonium wastewater. Small ammonium molecules diffused within the formed nitrifying biofilm and a surface biochemical reaction occurred. Nonetheless, the fixed biofilm enabled simultaneous conversion of accumulated nitrite to nitrogen. Fixed biocarriers provided more mass transfer in comparison of moving biofilm carriers or suspended growth systems [3]. According to literature, SND occurs in the depth of SBBR-PE biofilm carriers [47, 48]. The average MCRT for PE (Kaldnes) (10.0 days) was far much less than achieved when PU foam (22.9 days) was used. For NH_4^+-N and COD, thin biofilms (Kaldnes) actually do better at lower organic load values (<2.5 gCOD/m^2·d), with stable output at medium organic load values [81].

5.1.1.3 Polypropylene (PP)

As with most thermoplastic biocarriers, PP biocarriers just couldn't get the biofilm prepared for COD removal in an SBBR during the early days due to its smooth surface, which made it hard for microbes to bind, and in the meantime, when the water was pumped in and out, the biofilm could easily be washed off [67]. The effluent COD with PP biocarrier was about 39~88 mg/L. High-efficiency phosphorus removal in the built and run moving bed sequencing batch biofilm reactor (MBSBBR) reactor [30] is likely to benefit from a combination of biofilm cultivation on BioBall® carriers and suspended biomass in the reactor. Even though, phosphate accumulating organisms (PAOs) may sometimes occupy the biofilm media. The evolution in the total quantity of attached-growth and suspended biomass involved in the reactor will contribute to the fluctuation in the nitrification rate in the MBSBBR since nitrification rates depends on oxygen penetration and the mixture of heterotrophs and nitrifiers inside the biofilm [21, 50].

5.1.1.4 Polyvinyl Chloride (PVC)

SBBR-PVC efficiency was examined by [51] in phosphorus and carbon removal. Sadly enough, clogging took place in the plastic module when COD_T average removal rates were 8.3 g COD_T m^2/d and 1.1 kg COD_T m^3/d. Analysis performed following the incidence of clogging did not show any substantial reduction in the unit's COD removal capability.

5.1.1.5 Acrylic plastic

An acclimatization time that was 2-3 d shorter than that of a traditional SBR system with 5-7% higher COD and BOD_5 removal efficiencies was obtained by the research team [8] using a sequencing batch reactor biofilm (MSBR) system. This could be supported by the fact that, there was an increased quantity of biofilm mass on the acrylic plastic media. Hence, the overall bio-sludge mass of the MSBR system was greater than that of the SBR system.

5.1.2 Granular Activated Carbon (GAC)

Employing GAC with additional biomass plays an excellent role in nutrients and heavy metals removal as adsorbent, filtration unit and biofilm attachment in wastewater treatment It demonstrated excellent efficiency in performance and portrays a reliable process of treatment under stern organic load variations [57, 59]. GAC have severally been reported to possess high adsorption capacity [61, 62].

To begin with, NH_3-N and COD demonstrated stable removal during the entire study duration by [54] using an multimedia-sequencing batch biofilm reactor (MM-SBBR). High suspended solid and color removal efficiencies were obtained using GAC with physical adsorption as the main colour removal mechanism [53, 60]. Other researchers also claim that, after treatment with the pilot GAC-SBBRR, it was almost hard to foresee 2,4-dichlorophenol (2,4-DCP) residues in the effluent [55, 56]. Results of the study by [58] was in the agreement with several other studies showing high COD removal efficiency. Even at higher OLRs without process inhibition, the GAC-biofilm sequencing batch reactor (SBGR) system retained its efficiency. The subsequent suspended growth system, however, led to process failure. This can be due to the existence in the reactor of high composite chemical wastewater substrate gradients that inhibit native biomass. Considering the above-mentioned significant performance, it can be concluded that GAC biofilm has the potential to be further developed into low cost and environmentally friendlier process as no chemical application is required with zero chemical sludge production.

5.1.3 Waste materials

The insertion of the inner tube of used tires could increase the moving biofilm aerobic sequencing batch reactor (MB-aerobic-SBR) system's efficiency even at high organic loading. This has been seen as MB-aerobic-SBR effluent consistency has been more stable at the same operating conditions than aerobic-SBR. According to [63], the BOD_5, TP and TKN removal efficiencies of the MB-aerobic-SBR were 1–2, 10–20 and 2–3% higher than aerobic-SBR, respectively. Given the porosity, hydrophilic and non-toxic characteristics of LS, it could provide great number of microorganisms both inside and outside of it with habitat for their growth and reproduction, MBSBR-LS could efficiently extract organic contaminants [67]. The COD and NH_3-N removal efficiencies are depicted in Table 2. The TN removal efficiency could be enhanced by the denitrification process and subsequently enhance external carbon availability in the biocarrier [66].

Iron shavings can be utilized as the principal substrate to support denitrification process in SBR operation. However, it may result to very low denitrification rate. TN removal is closely related to the total iron concentration in reactor. Effluent iron concentration recorded was as low as 2–4 mg/L and the depleted iron were mainly adsorbed over the microorganism's surface, thereby affecting the microbial activity. An effective way to regain microbial activity is the extraction of TSS by half and subsequently adding fresh mixed liquor suspended solid (MLSS) with extremely low iron concentration [68].

POC provides low cost and simple operation. It is said to be more efficient as COD managed to achieve the targeted effluent limit of ≈ 20 mg/L for the reactor with POC as a biocarrier. This proved that the application of POC in SBR mode helps in improving the effluent quality in wastewater treatment [64]. Just like the inner tubes of used tires, high OLR increases the biofilm total VSS ratio in a peach pit supported SBBR system [9]. Similarly, as OLR and NLR were increased with decreased HRT in a WAS pellet biocarrier supported system, the reactor performance gradually increased under with stable nutrient removals. These suggests that the biocarriers used are very efficient for biomass retention [29].

5.1.4 Steel disk

When a steel disk was used in SBBR system, the improved performance in the absorption of organic substrate leads to a high denitrification efficiency [70]. The thickness of the biofilm varies from 1-20 µm and allowed the organic substrate to be ingested effectively.

5.1.5 Clay minerals

5.1.5.1 Mineral coal (MC)

In an SBBR flow cell experiments, the production of shortcut nitrification-denitrification to achieve efficient nitrogen removal can be credited to the selection of MC as a biocarrier. This is dependent on the need for a lower volume of oxygen and organic sources to support the procedure [19]. For the initial experimental periods, high VSS and TSS removal efficiencies were reported. However, increased concentrations occurred in the subsequent periods, possibly due to biomass detachments [73]. A progressive decline in the efficiency of COD removal was also noted during the experiments.

5.1.5.2 Ceramic

The SBBR packed with Liapor ceramic spheres was at its best at less organic load [81]. The biocarrier increased nitrate concentration, which gradually decreased at the end of the run, with effluent COD levels between 17-20 mg/L. The highest TN values for backwashing were achieved, suggesting that greater quantities of biofilm were sloughed off during this process. In a related study, the addition of spherical ceramic biocarriers enhanced the removal of TN and TP. The improved efficiency of nutrient removal was mainly because of biofilm bacteria and algae development on the biocarriers. Moreover, the sludge discharge and biocarrier replacement were independent, suggesting that algae and sludge retention time could be segregated. The harvesting of accumulated algae in biocarriers will sustain the quantity and operation of algae biomass, which is good for the long-term stability of the A-SBBR system [74].

5.1.5.3 Inert stone chips

At steady state condition, SBBR operated with inert stone chips showed high COD removal [20]. Biofilm floc immobilization on stone chips with an anoxic micro-niche in the inward sections may be due to sulfate conversion in the SBBR. According to the authors, at higher OLR, the reactor consistently showed good performance and stabilized within a short period of time.

5.1.5.4 Pumice stone

In an FB-SBBR, volcanic pumice stones were used as biocarriers. the reactor achieved steady state condition with a medium effluent COD concentration of 56.2 mg/L [16]. In a related study, synthetic pumice (SP) showed chemical constituents loss with a COD effluent of 233±52 mg/L during the reactor operational process [82].

5.1.5.5 Zeolite

Natural zeolite sequencing batch biofilm reactor (NSBBR) and synthetic zeolite sequencing batch biofilm reactor (SSBBR) zeolite filled SBR were used to assess the impact of NH_4^+-N adsorption ability on microbial characteristics and nitrogen conversion. Nitrification and ammonium exchange occurred concurrently with significant nitrite production rate differences observed in the two reactors studied by [72]. NOB was successfully inhibited while effluent NAR was average at 95%. This shows that the reactors will both accomplish PN. Zeolite plays the dual functions of biomass carrier and ion exchange for NOB and AOB during adsorption with high selective NH_4^+-N adsorption potential. This in turn made SSBBR and NSBBR differ in FA concentrations. AOB in the SSBBR desorbed the high NH_4^+-N concentration, because its NH_4^+ -N adsorption capacity was 2–3 times larger than that of NSBBR.

5.1.6 Fibrous biocarriers

Achieving good TSS and COD removal efficiencies, SBBR coupled with the flexible fibre packaging material will successfully yield excellent result. There is an inverse relationship between the efficacy of COD removal with regard to changes in OLR. However, a direct relationship exists in the case of TSS performance [83]. The high initial concentration of COD could lead to the biofilm absorption of aerobic organic matter. Thereby, transporting organic matter into the internal biofilm, to serve as a source of carbon during denitrification [87]. Therefore, SBBR systems developed with fibrous materials as biocarriers are highly efficient and energy saving wastewater treatment technologies. It is apparent that SB-FFBR can withstand OLR to a certain degree and can handle very high contaminated wastewater. However, with an increasing organic loading rate, the COD dissolution rate shows a rapid decrease. The reduction in COD removal ability with an increment in OLR in the SB-FFBRR could be due to the decrease in contact time between microorganisms and substrates [78]. Similar to most biocarriers, the crucial determinant for attaining high COD removal efficiency at the most leading OLR was the increased amount of biomass concentration. This was due to the existence of fibrous packing media that provide a more precise surface area for the growth of microorganisms. A research team in their study [75], attained low NH_3-N removal efficiency. This result revealed that autotrophic bacteria activity was inhibited by the concentration of carbon and that the efficacy of biofilm-absorbed aerobic organic substances was simpler than the absorption of NH_3-N.

Increasing the biofilm density of a fibrous biocarrier from 15 to 50% resulted in greater biomass volume. This show that the number of microbes has increased, resulting in low NH_4^+-N, TN and COD effluent concentrations. However, the high biomass inhibited mass transfer as the biofilm density proceeded to rise from 50 to 70% and the microbes were unable to access the organics. Thus, this affects the pace of COD elimination. Due to the large number of microorganisms and consistent influent concentration, the average nitrogen load per biomass was also low, thereby reducing TN removal rate. Hence, these two studies [1, 10], recommended biofilm density of 50%. In an SBBR system made of rope-type media, the cyclic COD profiles showed that most biodegradable organic compounds were absorbed in the anoxic process by facultative bacteria leaving the non-biodegradable component during the rest of the cycle period [86]. Similar to other biocarriers, the increase in COD load seems to have resulted in high heterotrophic bacteria growth rates during the liquid phase of the reactor. As it showed appropriate volumetric removal rates of total inorganic nitrogen, the fixed-bed biofilm sequencing batch reactor (FbSBR) was regarded as an efficient and portable mechanism.

5.1.7 Composite packing

In the experiment conducted with a sequencing batch biofilm reactor combined with a vertical flow constructed wetland (SBBR-VFCW), the VFCW performed well in NH_4^+-N dissolution. Plants and Clinoptilolite and in the VFCW also demonstrated strong NH_4^+-N absorption [14]. The SBBR-VFCW system could occupy less space, work stably, run normally, cope with higher hydraulic loads, withstand impact loads and obtain greater purification. Combination of GAC and natural zeolite as biocarriers in the anaerobic sequencing batch biofilm reactor (ASBBR) improved biogas generation and COD removal rate. Observed effluent COD concentrations was 30-40 mg/L with 278 mL/d as the maximum biogas productions. Authors reported that GAC was responsible for the improved COD degradation while zeolite was for gas generation [71]. The high biomass concentration within the moving-bed biofilm sequencing batch reactor (MBBSBR) was due to the efficiency of the composite biocarrier in the aggregation and immobilization of bacteria. This ensured the greater performance of treatment through high loading and allowed shock loading to be strongly tolerated. Although the efficiency of COD declined by 8%, study also reveals that microorganisms have increasingly adapted to the toxic environment after a pretty long operation and have lived on organic compounds found in wastewater [65].

5.1.8 Comparative analysis on nutrients and organic matter removal efficiencies

The experimental results were compared based on treatment efficiency achieved due to the influence of different biocarriers applied. From Table 3 below, it could be observed that PU, GAC, POC, inert stone chips, and biocarriers made of composite packing achieved high and stable COD removal efficiencies. In some cases, even at high OLR values. On the contrary, PP, MC, Fibrous biocarriers had low COD removal rates with some even in a decreasing trend. The utilization of PE, LS, MC, ceramic as biocarriers result to high TN removal. This could be due to biofilm bacteria and algae development on some of the biocarriers which enhance the denitrification process. But when PU was used, low TN removal was recorded due to large PU size. NH_4^+-N removal efficiencies in SBBR supported with PU, PE, GAC, Zeolite, and biocarriers made of composite packing for organic and nutrients removal alongside biofilm formation were high due to the exceptional properties possessed by the biocarriers. Fibrous biocarriers had low NH_4^+-N removal. Used tires removed TKN, BOD_5, and TP at high efficiency as SS and TSS were highly removed by GAC and Fibrous biocarriers respectively. PP and ceramics also removed TP at high efficiency.

Table 3: Comparative performance of various biocarriers in organic and nutrients removal

Biocarrier	Performance
PU	• At higher organic loads, better removal of COD and NH_4^+-N could be attained. • Promotion of SND processes alongside an improved TN removal. However, TN removal decreases with decreasing PU foam cube sizes while NH_4^+-N removal decreases with increased PU foam volume • The removal of NH_4^+-N may also be credited to the reality that the high pores of the PU biocarriers have been trapped by many microorganisms
PE	• Highly efficient nitrogen removal was achieved. • Nitrite reduction was also observed. • High TN and NH_4^+-N removal
PP	• They just couldn't get the biofilm prepared for COD removal during the early days due to its smooth surface, which made it hard for microbes to bind. • High-efficiency phosphorus removal
GAC	• Even at higher OLRs, NH_3-N and COD demonstrated high and stable removal • High SS and color removal efficiencies could be obtained
Waste materials	• Inner tube of used tires improved BOD_5, TP and TKN removal efficiencies. • The TN removal efficiency could be enhanced by the denitrification process and further enhance external carbon availability in the LS. • POC is said to be more efficient as COD managed to achieve the targeted effluent limit
MC	• Achieved efficient nitrogen removal due to the biocarrier need for a lower volume of oxygen and organic sources to support the experiment. • A continuous reduction in the efficiency of COD removal.
Ceramic	• Enhanced removal of TN and TP primarily due to biofilm bacteria and algae development on the biocarriers
Inert stone chips	• High COD removal even at higher OLR
Zeolite	• High selective NH_4^+-N adsorption potential
Fibrous biocarriers	• Excellent result for TSS removal • COD deceases at high OLR • Low NH_4^+-N removal
Composite packing	• High NH_4^+-N and COD absorption • Improved biogas generation • Occupy less space, work stably, cope with higher hydraulic loads, withstand impact loads and obtain greater purification

5.1.9 Biocarriers significant effect on the removal of hardly biodegradable compounds

Most hardly biodegradable compounds affect SBBR system performance at high concentration during initial system operational stage, irrespective of the biocarrier material type. Their presence reduces microbial activity and affects microbial community structure, diversity and composition. The protein and polysaccharide contents in the EPS of the biofilm and suspended sludge increases with an increase in hardly biodegradable compounds concentration. Their addition in the influent could affect the chemical structure of the LBEPS and TB-EPS.

Researchers [55, 56], reported that, after treatment with the pilot GAC-SBBRR, it was almost hard to foresee 2,4-dichlorophenol (2,4-DCP) residues in the effluent. A study [54] using same biocarrier material treating *pentachlorophenol* (PCP) also reported good performance efficiency and stable treatment process under stern organic load fluctuations with about 61% PCP removal. Utilizing a rotating perforated tube as a biocarrier material increases DCP removal efficiency. This was proven by the large biofilm surface area at high A/Q ratios. Interestingly, increasing DCP feed concentrations improved DCP removal efficiency up to > 97% [89].

According to the findings of this review, it was discovered that two biocarriers were most prominent when handling hardly biodegradable compounds. These are: (i) Fibrous carriers and (ii) combined packing that made of polyester fiber. The fibrous biocarriers have been used by researchers to treat wastewater with significant concentrations of oxytetracycline [90], chlortetracycline [91], sulfadiazine [92], florfenicol (FF) [93], norfloxacin [94] etc. compared to several other biocarriers, the fibrous material has high resistance to toxic effects resulting from the aforementioned compounds. Combined packing made of polyester fiber materials acting as carriers for microorganism attachment have been utilized by several researchers specifically treating wastewater containing different proportions of cerium oxide nanoparticles (CeO_2-NPs) under both short and long-term exposure conditions. It was reported that CeO_2-NPs were adsorbed as aggregates as low concentrations did not affect the biofilms, and the resulting ROS production increase influenced cell growth [95, 96]. The inhibitory effects of CeO_2-NPs with molecular oxygen were reduced after anaerobic exposure [97].-At the end of aerobic exposure, the contribution of EPS to phosphorus removal improved, while the capacity of EPS in phosphorus storage declined after exposure to CeO2 NPs [98].

Table 4. Efficiency of biocarrier materials for nutrients and organic matter removal in SBBR

System	Reactor Capacity (L)	Biocarrier type	Wastewater type	Loading rate	Nutrients and organic matter removal (%)			Specific area	Filling ratio	Ref.
					COD	NH_4^+-N	TN			
SBBR	31	Linpor (PU)	municipal wastewater	0.5-8.0 gCOD/m^2·d	93			270 m^2/m^3		[81]
		Kaldnes (PE)			73.83			500 m^2/m^3		
		Liapor (Ceramic)			92.06			480 m^2/m^3		
ASBBR	1200	MC	industrial wastewater	OLR, 0.6-3.0 kgCOD/cycle; COD_{Total}, 0.98-5.08 g/L; NH_4^+-N, 76-515 mg/L	86				0.83	[73]
FB-SBBR	15	MC	NH_4^+-N rich wastewater	0.55±0.10 kgNH_4^+-N/m^3/d			95		0.52	[19]
AnSBBR	7.2	PU	domestic sewage	COD, 301±103 mg/L	60			43.8 m^2/g	0.51	[82]
		VC		COD, 347±87 mg/L	40					
		SP		COD, 446±169 mg/L	48					
		PE		COD, 337±82 mg/L	33					
SBBR	35	PU	synthetic wastewater	0.24-1.26 g N/(L·day); COD, 372-382; NH_+-N, 39.8-209.5 (mg/L)	86.88	74.6	60.5		0.8	[37]
SBBR	10	PU	domestic wastewater	51–85 mg N/L/d	77.44	99.44	88.2		0.2	[44]
ASBBR	5.5	PU	synthetic wastewater	COD, 1000 mg/L	78-83					[42]
MBSBRs	12.8	PU	synthetic wastewater		70		84	177 m^2/kg	0.08	[39]
MBSBRs	12.8	PU	synthetic wastewater		64		59.5	177 m^2/kg	0.08	[41]
AnSBBR	10	PU	synthetic wastewater	0.4 and 3.2 COD/SO_4^{2-}	67			43.8 m^2/g		[79]
		EC			81			3.51 m^2/g		
SBBR	10	PU	synthetic wastewater	0.78-1.7 kgN/m^3/d			93	2395 m^2/m^3	0.15	[33]
ASBBR	7.2	PU	cane vinasse	OLR, 0.5-5 g/L.d, 9.6 gCOD/L	70					[43]
MBSBRs	8	PU	synthetic wastewater	NH_4^+-N, 50 mg/L		99	71	900 m^2/m^3	0.2	[66]
		LS				99	78	850 m^2/m^3		
SBBR	10	PU	synthetic wastewater		80	100			0.02	[80]
		PE			76.59	96				
SBBR	28	PE	autotrophic medium				75	407 m^2/m^3	0.47	[49]
MBSBR	6.5	PU	municipal sewage	COD, 213-286; NH_4^+-N, 21.1-28.5 mg/L	92.43	97.89	83.73	>20,000 m^2/m^3	0.3	[40]
ASBBR	1200	PU	domestic sewage	0.2–1.2 kg COD/m^3 /d_	62					[38]
SBBR	2	PE	diary wastewater	1130-1560 gBOD_5/m^3.d	81.8	85.1		150 m^2/m^3	0..3	[17]

SBMBBR	5	PE	synthetic wastewater	COD, 649.44 mg/L	97				0.05-0.1	[4]
ASBBR	3.4	PE	synthetic wastewater		94.8		95.0	500 m^2/m^3	0.4	[27]
SBBR	9	PE	municipal wastewater	500-1000 mg/L COD	90	68.2	60.1	460 m^2/m^3	0.35	[31]
FB-SBBR	14	PE	synthetic wastewater		95					[16]
		Volcanic pumice stone			92.9					
SBBR	30	PE	synthetic wastewater	0.5-2.3 kg of COD/m^3.d	99	99		690 m^2/m^3		[47]
SBBR	30	PE	synthetic wastewater	COD, 1460±2; NH_4^+-N, 265±2 (mg/L)	93.7	99.1	97.5	369.6 m^2/m^3	0.4	[48]
MB-SBBBR	5.5	PE	acid Red 18 (AR18)		83.07			415 m^2/m^3	0.5	[45]
SBBR	20	PE	pharmaceutical wastewater	COD, 10000 - 10050; NH_4^+-N, 1875 - 2000 (mg/L)		98.4		480 m^2/m^3	0.23	[3]
SBBR	3.4	PE	ammonia wastewater		99.6	98.2	61.2	500 m^2/m^3	0.4	[18]
SBBR	28	PE	poly-b-hydroxybutyrate			66		407 m^2/m^3	0.47	[46]
IFAS-SBR	800	PE	municipal wastewater	COD, 150-500; NH_4^+-N, 50 (mg/L)	98.6	99		800 m^2/m^3	0.5	[26]
SBBR	27.6	PVC	domestic-strength synthetic effluent	8.8 g COD_T m^2/d and 1.2 kg COD_T m^3/d	94			240 m^2/m^3		[51]
MBSBBR	15	PP	synthetic wastewater	0.84–0.978 g COD/L/d; 0.074–0.106 g N/L/d	97.7		87.8	378 m^2/m^3	0.1	[30]
SBBR	19.8	PP	sewage treatment		83	80			0.5	[67]
		LS			89	90				
SBMABR	2.6	PP	synthetic wastewater		90	96	91			[50]
SBR-BF	1000	PP	urban sewage	NH_4^+-N, 35; TP, 13; COD, 400 (mg/L)	95		94		0.3	[21]
GAC-SBBR	2200	GAC	recycled paper wastewater	700-1000 mg COD/L	97.2	99.4				[55]
GAC-SBBR	2200	GAC	recycled paper wastewater	0.7 OLR (kg COD/m^3.day).	94	98				[56]
MM-SBBR	18	GAC	paper mill wastewater	750-1900 mg COD/L	98	100				[54]
GAC-SBBR	5	GAC	recycled paper mill wastewater	800-1300 mgCOD/L	99	94				[53]
HG-SBR	7	GAC	synthetic wastewater	632 ± 50 mgCOD/L	96					[59]
SBCR	2	GAC	dye-containing wastewater	COD, 250-2500; C.I. Acid Orange 7 (AO7), 125-625 (mg/L)	88					[60]
Expanded bed biofilm reactor	7	GAC	zinc and copper containing wastewaters	COD, 3000; Zn, 200 (mg/L)	90					[57]
Spent GAC–biofilm-SBR	1.8	GAC	methyl orange (MO)-wastewater	COD, 541; MO, 175 (mg/L)	86					[61]
GAC–biofilm SBR	1.8	GAC	diazo dye Reactive Black 5 (RB5)-	COD, 467; RB5, 200 (mg/L)	94					[62]

SBGR	1.7	GAC	chemical wastewater	OLR: 1.7 kg COD/cum-day	78					[58]
SBBR	5	Steel disk	synthetic wastewater	TN, 7-140; NO_3^-N, 7 mg/L			99			[32]
MB-aerobic-SBR	10	Inner tube of tires	synthetic wastewater	COD, 450±49; TP, 5.0±0.55 (mg/L)	97.5					[63]
SBBR	2	Iron shavings	artificial wastewater	COD, 100±5; NO_3^--N, 41.5±1.5 (mg/L)			90.36	0.002634 m^2/g	0.7	[68]
SBBR	10	POC	synthetic wastewater	COD, 50-100; NH_4^+-N, 4-8 (mg/L)	67	90			0.4	[64]
FSBR	12	Peach Pit	synthetic wastewater	OLR, 2–12 kg COD/m^3/d	98.77					[9]
SBBR	2.5	WAS pellet	synthetic wastewater	NLR, 120; OLR, and 60 (g/m^3-d)	93	99	95			[29]
SBBR	4	Ceramic	domestic wastewater		90		65.8	900 m^2/m^3	0.2	[74]
SBBR	1.4	Inert stone chips	chemical wastewater	0.92- 4.76 kg COD/cum-day	88.05				0.49	[20]
SBBR	18	Fiber threads	domestic wastewater		90					[75]
SBFFBR	8	Rayon fiber	dairy wastewater	OLR, 0.38-2.74 kg COD m^3/d	97.2			2200 m^2/m^3	0.9	[83]
SBBR	5.3	Semi-soft fiber	municipal wastewater	COD, 192.82; TN, 57.34; NH_4^+-N, 51.37 (mg/L)	85	94.2			0.7	[1]
ASBBR	2.4	semi-soft fiber filler	mustard tuber wastewater	COD, 3600-8200; TN, 560-1100; TP, 8-19 (mg/L)	91.3				0.7	[10]
SB-FFBR	8	Flexible fiber	milk process wastewater	OLR; 0.38–8.19 gCOD/m ^{3}/d	97.5			2200 m^2/m^3	0.9	[78]
FbSBR	21	Rope-type media	piggery wastewater	COD, 2000; NH_4^+-N, 350 (mg/L)	85		80			[86]
SBBR	24	Porous polymer	Livestock and Poultry Breeding Wastewater	COD, 1000–2500; TN, 125–175 NH_4^+-N, 85–123 (mg/L)	96.1		92.1	5357 m^2/m^3	0.05	[52]
HSBR	1420	Nylon nets	municipal sewage	0.51 kg COD/m^3.day and 0.06 kg NH_4-N/m^3.day	92	99		4140 m^2/m^3		[87]
SBBR	0.5	Aluminum foil	synthetic wastewater	NH_4^+-N, 100.4 ± 1.5 mg/L		93.7	77.5	500 m^2/m^3		[88]
SBMBfR	2	Fibrous slag	synthetic wastewater				96			[84]
MSBR	25	*Acrylic plastic*	milk industry wastewater	680 g BOD_5/m^3 d	97.9					[8]
SBBR-VFCW	60	PVC, soft PE and porous aggregates	domestic wastewater	COD, 276.20; TP, 11.15; NH_4^+-N and TN, 79.25; (mg/L)	97	98.5	91.5			[14]
ASBBR	1	GAC and natural zeolite	synthetic municipal wastewater	COD, 1000 mg/L; OLR, 400 g/m^3 d	98					[71]
FSC-SBBR	10	PU and two fiber balls	synthetic wastewater	COD, 250–380; NH_4 $^+$-N, 40–50; TP, 10–15 (mg/L)	93.39	96.66		2.15-2.21 m^2/g	0.6	[85]
MBBSBR	2	PE and inorganics	coking-plant wastewater	OLR, 0.449 kgCOD·m^3/d	92.9			900 m^2/m^3	0.3	[65]
SBBR	9.7	Activated carbon fiber filler	swine wastewater	COD, 4684; NH_4^+-N, 846; TN, 1062; TP, 31.9 (mg/L)	98.2	95.7	95.6			[76]

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5.2 Biocarrier influence on biofilm formation

It is clear that biofilm configured systems are best fit for the remedy of wastewater comprising poorly degradable mixtures [58]. Apart from hydrodynamic conditions and substrate loading rates, the properties of different biofilm carriers hold important implications concerning biofilm maturity and in situ sludge minimization. The biocarrier concentration is obtained based on the percentage computed from the proportion of the carrier media's capacity to the reactor effective capacity [80]. In addition, biocarriers set on the water accumulator can not only fasten abundant microorganisms, but maintain the filtration function, letting the solid-liquid separation easier. To predict biomass adhesion and inceptive biofilm development on supports, surface tension parts of all supports and biomass is required to be computed by employing experimental values of contact angles [49]. Filamentous bacteria are important components for biofilm formation. However, different processes such as abrasion, erosion, sloughing and predator grazing can lead to their detachment [25]. The growth of films on the biocarrier cover can be evaluated utilizing a flow cell device [19]. Therefore, this sub-section would focus on the effect of various biocarriers in biofilm formation.

5.2.1 Thermoplastic

5.2.1.1 Polyurethane (PU)

The appearance of PU foam cubes as biocarrier can create an integrated aerobic, anaerobic and anoxic microenvironment, thereby providing ensnared carbon substrate that stimulates the SND process [39]. Aerobic conditions are usually retained on the surfaces of the PU foam cubes as DO inclination befell along the PU foam's interior depth [66, 80]. PU is extremely suitable in anaerobic digestion during biomass immobilization [82]. Therefore, understanding the recurrence and diversity in the microbial morphologies in the PU foam cubes associated biofilm [38].

Biofilm formation in an MBSBR was successfully achieved by [40], due to the porous nature of the biocarrier (PU foam) utilized, enabling small molecules such as oxygen to enter through the pores within the biocarrier and making the biofilm have distinct structures and micro-profiles . Water also entered the pores, presenting a way to convey substances inside the biocarrier to support bacterial growth. This could be due to the fact that the thick porous layer was able to effectively trap and capture biomass, and by having a large surface area, the reticulate structure facilitated biofilm accumulation. To further improve and sustain biofilm formation using PU foam, limited aeration was provided to create a favorable condition for biomass holding and a single oxic-anoxic interface for the enhancement of slow-growing anammox bacteria in an SBBR system [44]. Attached-growth biomass was the prevailing sludge morphology formed on the surface of biocarriers as depicted in Fig. 21. AOB and anammox bacteria development was successfully enhanced in the system. This could be attributed to the application of PU as a biocarrier and subsequently great nitrogen removal effectiveness of the reactor. Similar to findings by [37], NOB, *Nitrospira* and *Nitrobacter* gradually increased through the operational period. Even though, the Maximum NOB activity was limited to approximately 1.1 mg N·g/VSS/h [44].

An increase in biofilm biomass with concentration varying from 0.30 - 0.55 g TVS/g foam was noticed from the formulation of biopolymers (EPS) when [38] studied the efficiency of an ASBBR in the management of domestic sewage subjugated by anoxygenic phototrophic bacteria. The EPS could be responsible for the enhanced biofilm adhesion to the inorganic matrix. More so, the presence of fluorescent roads, anoxigenic phototrophic bacteria and other archaeal populations with any methanogenic organisms like *Methanosaeta* and *Methanosarcina*-like cells were observed at an oxidation–reduction potential ranging within -280 and -360 mV. From the perceived great frequency of these phototrophic organisms inside the biocarrier interstitial spaces, it can be concluded that PU have cooperated as a second substrate and micro-traps improving the retention of microorganisms during the reactors release stages.

Investigating the microbial succession within an ASBBR treating cane vinasse [43], optical microscopy showed high microbial morphological diversity. Rods of multiple morphotypes and filaments commonly associated to anaerobic degradation pathway hydrolysis and fermentation processes have been frequently observed. It was also noted that the mechanically preserved micro-granules inside the PU were neither different when compared to their size nor their microbial morphotypes.

Fig. 21. Formation of biofilms surface on biocarriers in the SBBR (left), and biocarriers filled with biomass on (right) (Adapted from [44]).

The hydrodynamic attributes of ASBBR could be the result of these observations, that reduces the impact of substrate delamination and mass transference inside the biofilm. The prevalent archaeal community inside the PU matrices was *Methanosarcina*-like microorganisms at high organic loading ratios beyond 3.3 gCOD/L.d. This has been proposed to be correlated with a higher specific rate of microorganisms' acetate intake. In a related study, when an ASBBR was filled with PU foam for treating sulfate-rich wastewater [79], the key organic matter was transformed by complete- or incomplete-oxidizing sulfate-reducing bacteria (SRB), in supplement to acidogenic microorganisms, without the use of sulphate as the electron donor. SRB was found to represent > 65% of the bacterial community.

Microbial community similarity in an SBBR operated by [37], decreased with the increase of NLR. The sequencing outcomes confirmed that, with two complicated biofilm systems in the microbial ecology, nitrogen extraction was accomplished by *Nitrobacter sp., Nitrospira sp.,* uncultured AOB, *Pseudomonas fluorescens*, and *Pseudomonas butanovora*. Interestingly, the bacteria quantity on the biocarriers improved following inoculation because of progressive immobilization of huge bacteria over the PU foam.

SEM photographs depicted in Fig. 22 unveiled that the bulk of the spherical and elliptical bacteria were besieged by the EPS and bacillus or filamentous bacteria in the reactor enrichments in period I. However, in periods III and IV, few or even no bacilli and filamentous bacteria were detected, suggesting good bonding between the biocarrier and the bacterial populations. Still on the effect of NLR in a PU foam filled reactor, biofilm system in a denitrifying ammonium oxidation (DEAMOX) process had powerful adaptability to fluctuating loading rate. The huge organic matter contents in SBBR led to the growth of heterotrophic denitrifying bacteria on the exterior of biofilms. The biofilm slowly grew thicker and became red (Fig. 23c, d), symbolizing that anammox bacteria evolved. As the NLR increases, a coating of yellow-gray bacteria, known to be heterotrophic denitrification bacteria, started to cover the biofilm surface, while the interior layer remained red well in the biomass attached on the biocarriers. The biomass densities in biofilms risen from 1000 plus to 3819 mgVSS/L over the whole duration of operation. This symbolizes that a mature and stable biofilm had been developed on biocarriers [33]. The concentration of anammox bacteria in biofilms rose from 6.08×10^{9} to 1.00×10^{11} copies/g dry sludge by 2 orders of magnitudes, suggesting that anammox bacteria conformed more adequately to carriers, with the biofilm eventually approaching maturity.

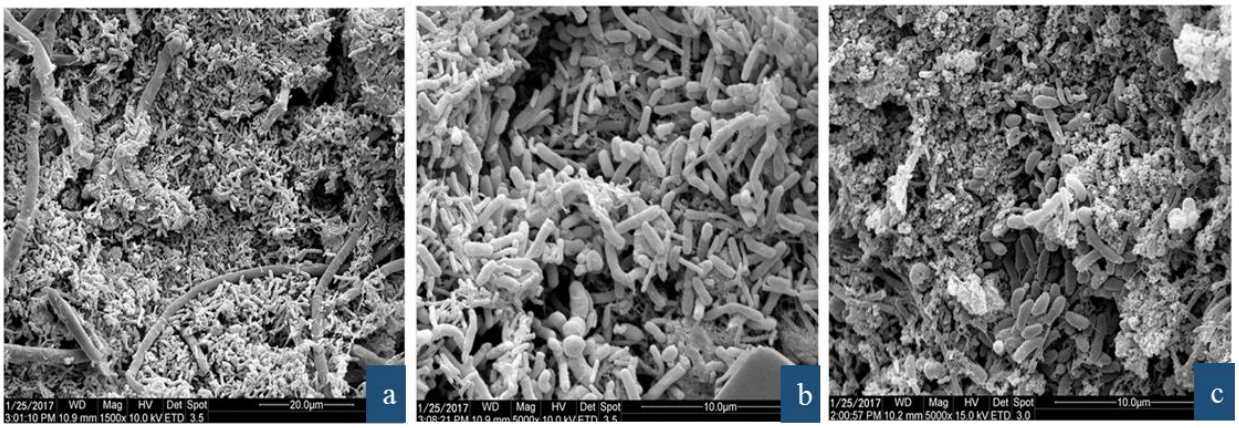

Fig. 22. SEM of biofilm grown on PU foams a, b, and c at different operational periods I, III and IV, respectively (Adapted from [37]).

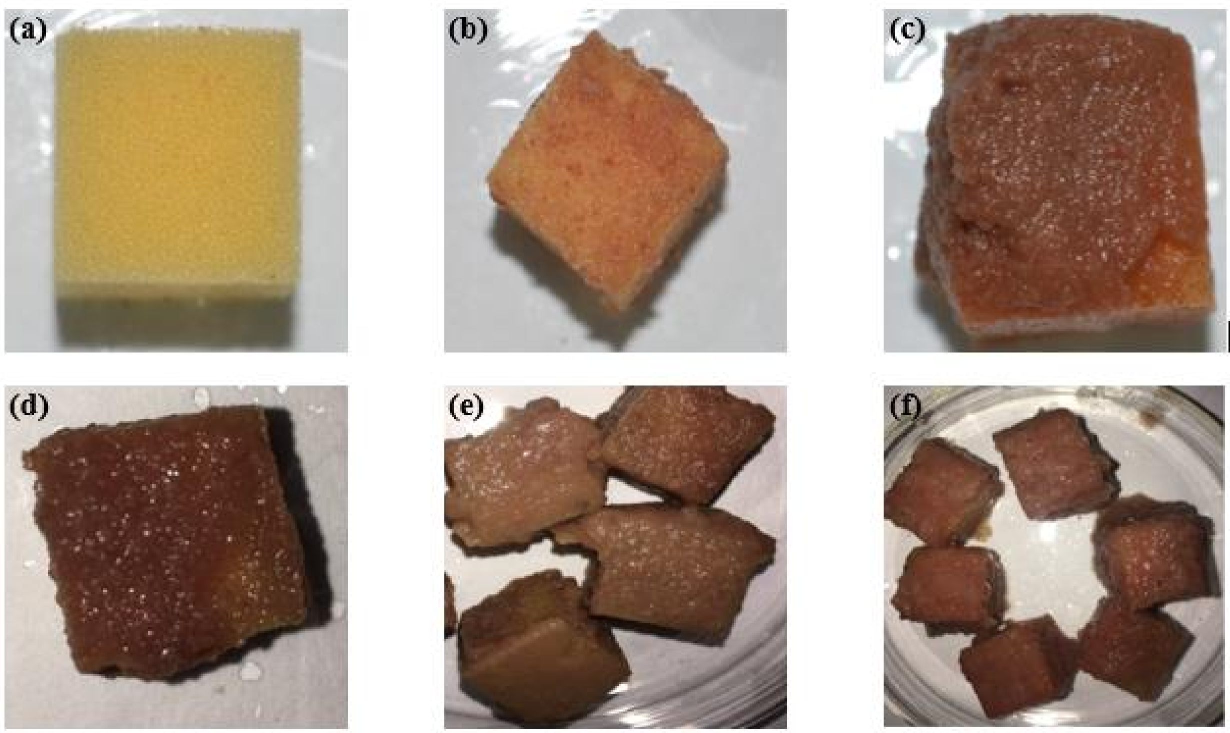

Fig. 23 Biofilm apparent morphology at different periods: (a) day 0; (b) day 9; (c) day 67; (d) day 91; (e) day 138; (f) day 196 (Adapted from [33]).

The effect of applying different biocarrier size in an SBBR have been extensively studied, because biocarrier size affects mobility of cubes to improve system performance [39, 41, 42, 49]. The effect could be better understood by studying the different mass transfer phenomena occurring in a biofilm reactor, namely: classic theory, intraparticulate convection theory, and convection inside the biofilm. The classic theory postulates that the smaller the particle, the better the external and internal mass transfer conditions. Intraparticulate convection states that the magnitude of this intraporous convection normally increases as biocarrier diameter increases. While, the theory for convection inside biofilm, state that, the biofilm structure may resemble a ‘‘tulip’’ or ‘‘mushroom’’, whose superior parts can coalesce, originating channels through which wastewater acting as nutrients can flow.

Maximum mass transfer could possibly be achieved when five phenomena (foam packing, liquid-phase mass transfer rate within the frame surrounding the bioparticle, intraparticulate diffusion, intraparticulate convection and internal fluxes in the biofilm) simultaneously occur in SBBR. Because of interior biofilm composition, outer liquid fluxes presumably affect the diffusion and convection that take place inside it; thus, the mass transfer inside the biofilm may be favored by an enhanced participation of convection in the macropores. Findings by [42] and [49] contradicts the classic theory as better mass transfer was experienced at higher particle size. This could be associated with the numerous magnitudes of the detachment forces in the reactors mainly induced by collisions within supports. It was also observed that, higher detachment forces are expected to occur in the reactors operating with smaller size biocarriers as higher quantity of support pieces might have stalled down initial biofilm accumulation.

5.2.1.2 Polyethylene (PE)

In an SBBR operated with PE as a biocarrier, biofilm can be distributed between the internal and external surfaces of the PE biocarrier material [46]. This is an advantage that PE biocarriers have over other biocarrier materials [3]. Also, among the several thermoplastic materials used for biofilm formation, PE has proven to be the most suitable bed material for the appendage of methanogenic microorganisms even though it has less satisfactory performance in nutrients removal [48]. Amazingly, PE can be pretreated to enhance its physical properties (surface area, roughness, and porosity). However, PE did not permit any form of microorganism to predominate [17, 27]. In tightly-bound biofilm, biofilm adhesion and compliance were the most substantial, with the weakest in supernatant/surface biofilm. According to [31], supernatant biofilm could be separated by lower centrifugal force (2000 ×g) while the tightly-bound biofilm require higher centrifugal force (10,000 ×g) to separate from biocarriers.

According to [82] in their study reported that a huge number of microorganisms is discovered on PE as it did not present adequate characteristics for promoting microbial growth. The relatively low porosity of PE did not permit for the appendage of an adequately huge concentration of VS on its surface to improve the craved performance. Hence, fit anaerobic biomass was discovered when testing PE as an immobilization support material. A study by [49] keenly observed that biofilm build-up was favored on the supports that granted tremendous interior surface area and shielded biofilms from erosion and abrasion separation mechanisms. The geometry of the collisions among particles and the hydrodynamic events instituted in the SBBRs appeared to perform a significant role in biofilm development than the thermodynamic synergy, expressed as free energy of adhesion (ΔG), among the biomass and support material. Other factors important in the biomass adhesion process, and are not regarded by the thermodynamic approach include: bacterial molecular structure and growth conditions followed by medium ionic force and composition.

The performance of PE biocarrier was also investigated by [47]. It was discovered that the dense particles inside the interstitial openings of the PE biocarrier were barely distinguishable from the biofilm fastened to the biocarrier surface as the huge biomass concentration in the bed manages to gather all the biomass. But, sampling a small volume of the bed and suspending it in water, it became evident that the reactor is made of two distinct fractions: the biofilm that lives fastened to the carrier and highly impenetrable aggregates (granules) that mechanically separated from the carrier. The PE biocarrier also aided biomass granulation through the following: (i) development of a faint biofilm that wholly masks the carrier; (ii) increment of biofilm thickness; (iii) break-up of the ascribed biofilm with the liberation of biofilm particles; and (iv) rearrangement of biofilm particles in smooth granules. Biofilm breakup can be caused by the continual increase in shear force that has significant adverse impact on biomass stableness. Biofilm is considerably impaired by an increment in shear force, because it develops with a pervious structure and little adhesion strength (18-22). Thus, resulting to biomass sloughing.

The positive role performed by the active biomass built as a biofilm on the coverings of the PE biocarrier media which beget a weaker responsiveness to toxicity impacts and other environmental provisions illustrates the divergent patterns exhibited by the SBBRs [80]. As depicted in (Fig. 24b and 24c), biofilm raised on coverings of the PE media had a fluffy arrangement. According to the authors [16], the microstructure of biofilm grown on PE when used as fixed-bed is different from that grown when used as moving carrier. Biofilm fastened to the moving carriers shows a profoundly permeable arrangement in which great number of filamentous bacteria lives. However, the biofilm appended to the fixed media had a compact arrangement with no filamentous bacteria noted.

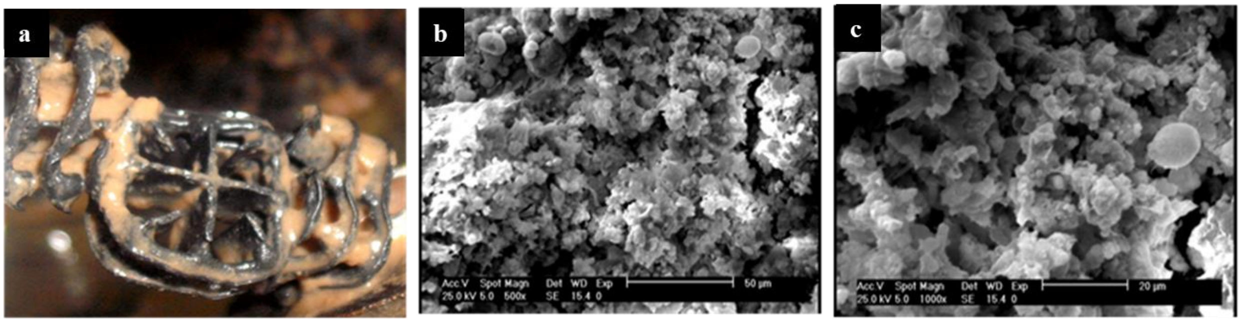

Fig. 24. Optical and SEM photographs of biofilm grown on surfaces of PE packing media in FB-SBBR (Adapted from [16]).

Similar to other studies that uses PE as a biocarrier, notable part of the entire biomass in an MB-SBBRs belonged to the attached-growth biofilm as the ratio of the biofilm to the suspended biomass concentration was achieved 0.51- 0.76 [45]. The SEM images of the biofilm raised on the coverings of moving biocarriers in MBSBBRs was obtained. As shown in Fig. 25a and 25b, after about 50 days, several surfaces of moving biocarriers were shielded by a brown layer of attached biofilm dominated by filamentous bacteria. It was also noticed that the community of filamentous bacteria was abundant in the suspended biomass in MBSBBRs.

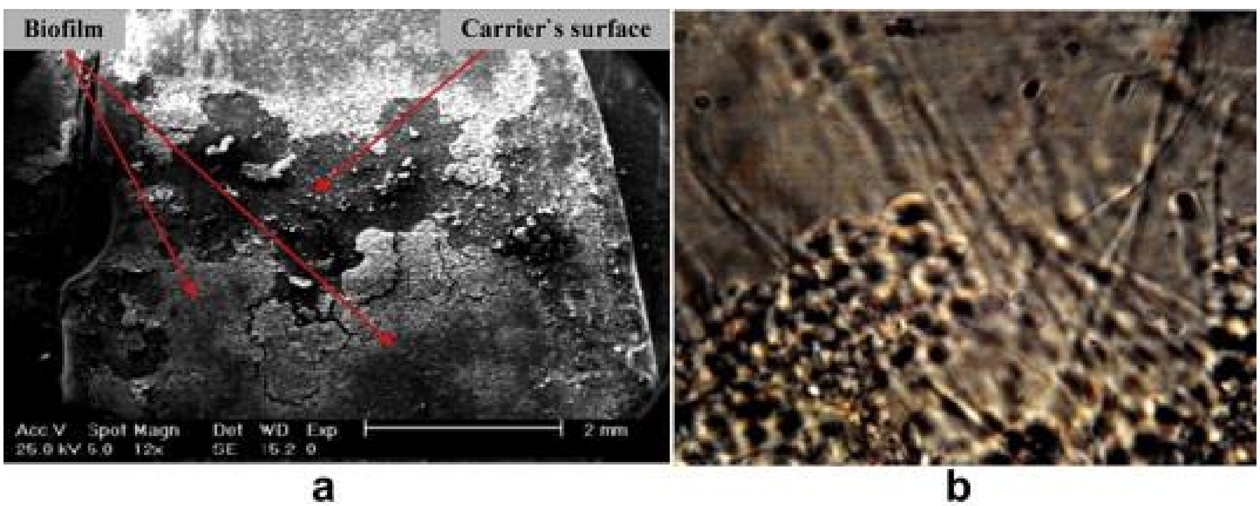

Fig. 25. SEM (a) optical microscopic (b) images of biofilm grown on surfaces of biocarriers in MB-SBBRs (Adapted from [45]).

Utilizing the Kaldnes (K_1, K_2, K_3, K_4, and K_5) form of PE encouraged nitrite production at the biofilm surface, inducing a huge concentration slope with an originally low nitrite concentration in the bulk fluid through nitrification. The expanding biofilm in Kaldnes exhibits features that make dispersion into the deeper layers hard. Thus, The composition of the biofilm influences the transfer of nutrients to and from the biofilm [81]. Unlike in PU foam cubes, biomass in the suspended flocs and in biofilm lowered as the organic loading reduces when PE biocarrier was employed. which demonstrated the consequences of the competition for oxygen and space among heterotrophic and autotrophic bacteria The percentages of nitrifying associated genes above cumulative genes remained higher in biofilms as correlated to those in AS flocs, symbolizing biofilm was a major favorable environment for nitrifiers [26].

During the biodegradation of mono azo dye-reactive orange 16 in sequencing batch moving bed biofilm reactor (SBMBBR), suspended-growth biomass controlled higher than 76% of the biomass system. The smaller dominance of attached-growth biomass herein could be associated to the: inoculated quantity of AS into SBMBBR; suspended biomass owns a more porous floc arrangement than attached biofilm. The SBMBBR system was confirmed to be capable of fighting mono azo dye-reactive orange 16 (RO16) shocks. However, a longer cyclic reaction time, higher biocarrier filling ratio, and post-treatment are needed to accomplish greater efficiency [4].

In a PN-SBBR system for nitrogen removal via nitrite treating high strength ammonia wastewater [18], the achievement of PN-biofilm was accomplished by variations in effluent quality and biofilm accumulation. Thin biofilm was easily attached onto the surface of PE biocarrier and ultimately, the biofilm thickness was commonly established in the system. Consequently, biofilm ambushed inside the biocarrier progressed to develop until stable operation of PN-system was achieved. At the end of the investigation, the cumulative quantities of biomass in the attached biocarrier and suspended phase were 5.35 and 0.5 g/L, respectively, insinuating that the larger share of the biomass was fastened over the biocarrier in PN-system.

5.2.1.3 Polypropylene (PP)

PP has been confirmed to possess a longer life span than other thermoplastic materials used as biocarriers in biological wastewater treatment [49, 67]. BioBall® renders a more dependable appendage medium than traditional plastic biocarriers for the maturity of microorganisms. In the study by [30], biofilm was evenly cultured covering the surface of BioBall® biocarriers, even though the layer was apprehended gradually due to the absence of microorganisms adhesion. The whole surface of the BioBall® media became shielded with a biofilm layer ≈1.2 mm thick.

The rise of biofilm thickness enhances the mass transfer resistance and decreases the internal mass transport efficiency of biofilm. Thus, backwashing process could be used to effectively control biofilm thickness. When backwashing process was employed by [50] in sequencing batch membrane-aerated biofilm reactor (SBMABR), the facultative aerobe and the anaerobic bacteria outside the biofilm did wipe off, while AOB inside the biofilm was properly stored. The little suspended microbes held in the effluent lead to the somewhat higher COD concentration discovered in the SBMABR system.

A hybrid system was successfully developed in an sequencing batch reactor-biofilm (SBR-BF) system with suspended and AS alongside attached biofilm [21]. In addition to rendering safe living circumstances for PAOs, denitrifying phosphate accumulating organisms (DNPAOs), nitrifying and denitrifying bacteria, the device has also restrained rivalry among different bacteria for carbon sources. Meanwhile, on suspended carriers, a coating of thin transparent yellow biofilm was noted.

5.2.1.4 Polyvinyl Chloride

It is a known fact that packed biofilms perpetually possess added openings. There is also a tendency for an increment in porosity with rising biofilm thickness. Authors [25], studied biofilm features and maturity with two tanks in an alternating pumped sequencing batch biofilm reactor (APSBBR) and confirmed that biofilm density decreases as the biofilm thickness increases in both tanks. The transformation of the biofilm density could also be due to varying morphologies and cell lysis. It was noted that the biofilm in Tank 2 was viscous and dense whilst a coagulated biofilm developed in Tank 1. Investigating the efficiency of a SBBR in organic carbon and phosphorus extraction, biofilm thickness increased with 96.2% as the medium moisture content of the biofilm. The highest total solids of the biofilm was 42.2 g/L [51]. Biofilm growth commenced on the module at the start-up of the reactor as the excessive growth made the module to block following long time work. This could be evaded if a larger amount of water was beyond the module.

5.2.1.5 Acrylic plastic

It was discovered that by placing an acrylic plastic media (2.7 m^2 surface area) at the base of an SBR system to get an MSBR system, the employment of an attached growth system will raise the quantity of biofilm piece on the media, increase the efficacy of extraction and persistence of sludge, decrease the quantity of surplus bio-sludge and also lessen the acclimatization time of the system [8].

5.2.2 Granular Activated Carbon (GAC)

In SBBR systems, GAC is being added as a biocarrier to support the production of biofilm as AS harboring microorganisms are sustained in suspension [53, 54]. The numerous benefits of using GAC as a biocarrier in SBBR systems are: long preservation of biofilm, suitable for treating poorly degradable compounds, provides les toxicant concentration in the bulk fluid due to sorption process, decreases biomass susceptibility to toxicants and successful increase of decolorization rate by increasing co-substrates concentration [58, 60, 62].

According to the findings by [57], the synergy amongst heavy metals and biofilm occurred in the adsorption of heavy metals upon biofilm, which slowly decreased the aqueous metal concentrations. More so, during the application of spent GAC by [61], the low reductive condition at the uppermost layer promoted the maturity of aerobic microorganisms and prompted the mineralization of medium aromatic amines. The GAC particles coated with biofilm were discovered to present a slight brownish colour [55]. Fig. 26 displays the optical microscopy as observed by SEM of the GAC molecules. Virgin GAC, with a dimension of 1.3 x 0.3 mm of porous structure, shown in Fig. 26 (a) and (b) offer niches of shelter from the accompanying shear forces and maintain surface biofilm [56]. It also contributed to the growth of microorganism and improved the GAC-SBBR's ability to extract COD and Adsorbable Organic Halides (AOX). The morphology of the biofilm shown in Fig. 26 (c) and (d) was discovered to be complex, with a staggering dense covering texture developed on the exterior of the GAC with a great interior permeable network.

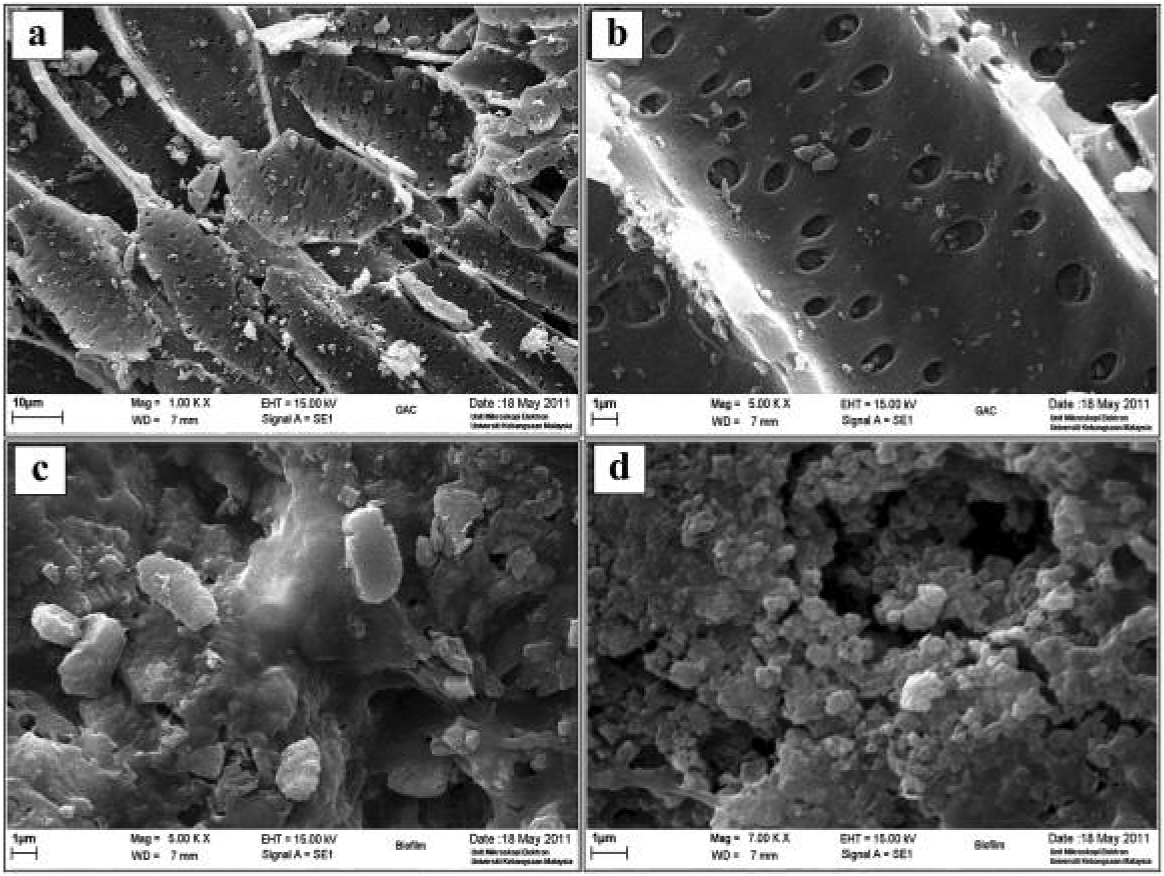

Fig. 26. Microscopic photographs for (a, b) virgin GAC and (c, d) biofilm GAC observed by SEM (Adapted from [55]).

A Hybrid-Growth Sequencing Batch Reactor (HG-SBR) system worked under immense absolute biomass concentration emanating from the production of bio-sludge and biofilm mass [59]. A positive relationship exists amidst MLSS and organic loading in HG-SBR. The increase in the values of MLSS and mixed liquor volatile suspended solid (MLVSS) was possible due to the growth of the microorganisms attached to the GAC. The co-existence of biofilm and bio-sludge enhanced the effectiveness of HG-SBR due to immense biomass array. Observation from the SEM images in Fig. 27 revealed that there stood a contrast amid the raw GAC and biofilm GAC. Cocci-shaped bacteria with bacterial diameter 0.25-1.0 µm prevalently built on the exterior of GAC biofilm (Fig. 27b) symbolized the creation of attached germination of microorganisms. A similar structure has also been noted in (Fig. 27c) alongside spherical and irregular shapes because multi culture microorganisms of AS were adopted during inoculation. The cocci-shape bacteria were nevertheless, the principal shapes that could be recognized.

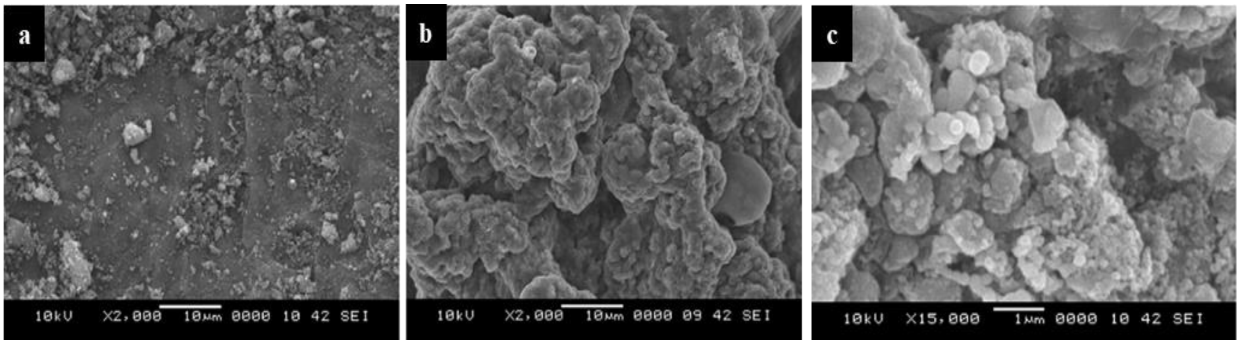

Fig. 27. SEM images for (a) raw GAC (b) GAC biofilm (c) bio-sludge microorganism (Adapted from [59]).

5.2.3 Waste materials

5.2.3.1 Inner tube of tires

The use of inner tube of tire as a biocarrier was explored. Biofilm was completely developed on the exterior of the media of the MB-aerobic-SBR with 1–2 mm thickness. The maximum biofilm volume on the surface was 17.18±0.7 g/m^2. This is about 30% larger than that of the conventional aerobic-SBR following the equivalent organic loading conditions. The other advantage of the MB-aerobic-SBR was the sustainable stability of effluent quality as early as 2–3 days during operation [63]. However, it must be noted that when utilizing tires inner tube tires, caution need be exercised to hold the metal-bound chemicals normally received in the tires. This can be done by carrying out chemical analysis. also, the leakage of hazardous materials, particularly heavy metals, into the effluent must also be tracked, throughout the operation of the system.

5.2.3.2 Luffa Sponge (LS)

As earlier described in section 4.3.5, LS is a natural biocarrier material used to simply capture a biofilm with fortified microbes throughout the early days of wastewater treatment. It forms biofilm faster than most thermoplastic biocarriers. The fast LS biofilm formation can be associated with the following factors: (i) high SSA which is hydrophilic (ii) enmeshment role of the 3-D fiber structure of the sponge can assemble microbial strains promptly (iii) biological compatibility made microbes develop and breed fast. Investigating the use of LS by [67], it was discovered that, the LS biocarrier had higher density than that of water after the biofilm was developed, since it was packed with large volume of active sludge and biofilm within and on its covering.

In a related study using MBSBR-LS, higher reduction was noted in nitrate-N, which had a lesser relative abundance of *Pseudomonas* (0.3%). The external carbon source produced by the breakdown of luffa, which could have led to the denitrification, might just have accelerated this [66]. Thus, it can be concluded that LS fiber biocarrier had a limited life span and could be progressively deteriorated by microorganisms, without causing secondary pollution.

5.2.3.3 Iron shavings

Unlike the LS biocarrier, biofilm formation was not observed on iron shavings surface during the early days of SBBR operation. In fact, the surface turned black after operating for a long period of time. Surprisingly, after 7 days of service, a thin biofilm started to develop on the reactor's internal surface with gradual increase in thickness. Unfortunately, the biofilm does sweep away as iron delivered by corrosion somewhat suspend in the effluent reactor and slightly absorb to biofilm. When the level of iron in suspended solid was > 70%, the sludge operation was severely suppressed and could be restored by greatly reducing iron concentrate in the reactor [68]. This easily elucidates that iron shavings are not suitable for biofilm formation.

5.2.3.4 Palm oil clinker

An investigation carried out by [64], reported that POC attachment improves the surface contact area with influent wastewater for biomass. Hence, the efficacy of rehabilitation and removal increased. The study, however, note that, a decline of the MLSS content during the sampling times was experienced which could be because of the biomass washout and the stagnation of biomass inside the POC biocarrier.

5.2.3.5 Peach Pit

Smooth biofilm without pores and channels were fully developed on the coverings of peach pits used in fixed-bed sequencing batch reactor (FSBR). This implies that the biocarrier was covered with a suitable biofilm, that elevated organic matter oxidation and diffusion into the biofilm [9]. At high OLR, the biomass activity in the biofilm secures great treatment capability and operational steadiness of the FSBR process. This proves that the use of peach pits as a biocarrier medium can be efficacious in the management of increased nutrient loaded wastewater.

5.2.3.6 WAS pellet

The introduction of WAS pellet into the reactor as a biocarrier to encourage microbial development was profoundly effective in sludge holding. Sludge units proved the co-existence and advancement of anammox bacteria, AOB and denitrifying bacteria in the reactor and biofilm production on to the biocarriers. The cumulative volume of biomass appended to the biocarriers signifies about 73% of the suspended biomass. This implies that the principal part of the biomass was fastened to the biocarrier as diverse classes of microorganisms immobilized and grew on to the biocarrier to produce biofilm [29]. Biocarrier saturation with fastened biomass and raw biomass continuously developed in the suspended stage proved that MLSS and MLVSS have substantially increased.

5.2.4 Steel disk

Research team by [69] in their study explored the application of steel disc in an AnSBBR. They discovered that organic substrate and nitrates were just accessible in the exterior layers of the disk due to the significant thickness of the biofilm. Bacterial cells presumably decayed in deeper and older layers of the biofilm harboring no substrates, thus the discharged organic compounds could be provided to exterior layers comprising nitrates.

In another article published by same authors [32], it was reported that biomass was mainly generated in the structure of a biofilm attached to fully submerged discs. The morphological structure of the biofilm reveals that biofilm thickness varied from 0.5-20 mm, because of the various impacts of abrasive forces. Despite portraying clear stratification, the film color varied from milk-like to dark brown. Bacteria of the biofilm in the submerged disk [70], manifested an improved intracellular polyphosphates growth. Also, the arrangement of the biofilm stimulated anaerobic–aerobic as well as anoxic–anaerobic and anaerobic conditions in a SBBR.

5.2.5 Clay minerals

5.2.5.1 Mineral coal (MC)

MC can be recognized as an effective inert supporter for biomass appendage, particularly for SRB and *methanogenic archeae*. It is a general observation that operational control for any situation can be realized following a concise time span following the advances in the operational circumstances, thus symbolizing the great capability of the MC to preserve biomass.

The experimental results for FB-SBBR during wastewater treatment with a high ammonia concentration demonstrated the potential of MC as a biocarrier. Authors [19], made this conclusion because of the high cumulative biofilm thickness (1954 μm) recorded, enabling the nitrification and denitrification to simultaneously befall. A study by [73] concluded that MC can be utilized for the simultaneous extraction of sulfate and organic matter provided ethanol is adopted as electron donor. However, at elevated doses, the development of TDS and COD has been noticed with increasing concentrations. This could be due to the immense concentrations of undissociated H_2S produced through the sulfate reduction process signifying that SRB were able to bind to the MC.

5.2.5.2 Inert stone chips

Treating chemical wastewater by inert stone chips coupled SBBR, authors [20] revealed that , stone chips coated with biofilm show sparse greenish brown color. Microscopic studies of the self-immobilized biofilm apprehended further revealed: (i) protozoa, fungi and bacteria emergence (ii) that the morphology of the biofilm possess an uneven surface texture with excellent interior permeable channels and (iii) heterogeneous with distinct biomass packs intermittently installed covering the surface.

5.2.5.3 Zeolite

Notwithstanding relating to varying alkalinity ratio, samples from natural zeolite (NZ) and synthetic zeolite (SZ) were alike. However, the population arrangement formed varied between the variable materials. The analysis proved that these modifications of the population arrangement were correlated with the ammonium adsorption potential of zeolite.

The variability could be caused by the distinctive adsorption ability of the materials, which regulate different FA in solution and farther affect the nitrifying microorganism movements [72]. The most prevalent genus in the NSSBR and SSBBR was *Betaproteobacteria* with the average abundances of 43.19% and 54.72%, respectively.

5.2.5.4 Pumice stone

In SBBR systems, SP presents well-matched anaerobic biomass, high substrate utilization rate and apparent first-order coefficient constant. However, it is not advisable to be utilized as a support material because of chemical structural damages. Authors [82], argued that, they realized a sudden boost of 82% in alkalinity when SP was used for immobilization. This increase could be clarified by the reaction of the SP with the substrate, which provided for carbonate synergies among them. Contrary to the behavior of SP, the prevalent bacterial strains in the biofilm cultivated on the scales of the volcanic pumice stones is spherical and rod-shaped bacteria, when used as a biocarrier in FB-SBBR1 [16]. The optical and SEM images of the biofilm formed on the packing material interfaces are presented in (Fig. 28a and 28b) where almost all the pores of the pumice stones used in FB-SBBR were masked by a panel of attached-growth biofilm.

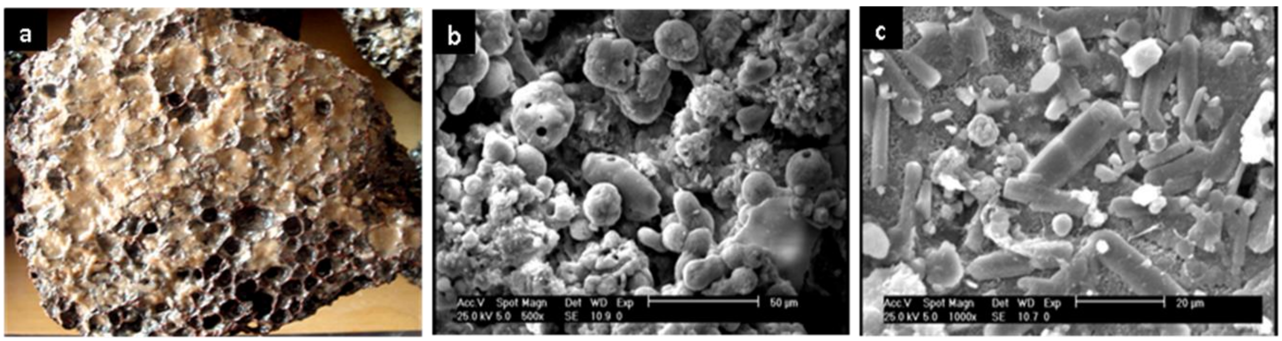

Fig. 28. SEM and Optical photographs of the biofilm grown on surfaces of volcanic pumice stones in FB-SBBR (Adapted from [16]).

5.2.6 Fibrous biocarriers

Fiber materials either flexible or semi-flexible serving as biocarriers possess huge SSA for microorganism appendage. Hence, bioreactors embedded with the fibers are able to treat high strength industrial wastewater because of the huge substrate and stock mass transfer rendered resulting from the fiber high SSA. They allow SBBR systems to withstand huge organic load and hydraulic shock loading because of synergistic associations between various microbial species developed in the biofilm [1, 10, 75].

A research group by [83] used flexible fibers in a sequencing batch flexible fibre biofilm reactor (SBFFBR) system for the wastewater treatment. They recorded an enhanced quantity of biofilm volume in the reactor leading to the elevated biomass concentration in the reactor. The main reason for the elevated biomass concentration in the reactor discharge phase may be the higher SSA of the fiber (2200 m^2/m^3). Another possible reason could be, the attachment of bulk biomass to the fiber biocarrier. According to findings as depicted in Fig. 29a, by same authors in a following paper after a decade [78], biofilms covered the surface of individual fibres and filled the space between closely opposed fibres. Clumps of bacteria surrounding and closely adherent to the individual fibre are observable in Fig. 29b. In the outer surface of the biofilm, a variety of bacterial morphologies; rod, coccal, spiral, and helicalmorphologies with a mixture of straight- and comma-shape rods were observed as the most abundant organisms (Fig. 29c and d). Identified by their tail-like structure, flagella were seen in (Fig. 29c) as the availability of DO attracted grazing nematode species (Fig. 29d).

An investigation focused on the viability of simultaneous nutrients removal by inserting a fibrous gas permeable membrane layer into a traditional SBRR. Authors [84] discovered that the technique permits long-term preservation of AOBs, allowing for the uninterrupted production of nitrite and nitrate that can be used by DNPAOs as electron acceptors soon after penetration from the biofilm into the bulk. However, the optimization of the membrane fibrous support, its cleaning, and the detection of DNPAOs linger as a problem.

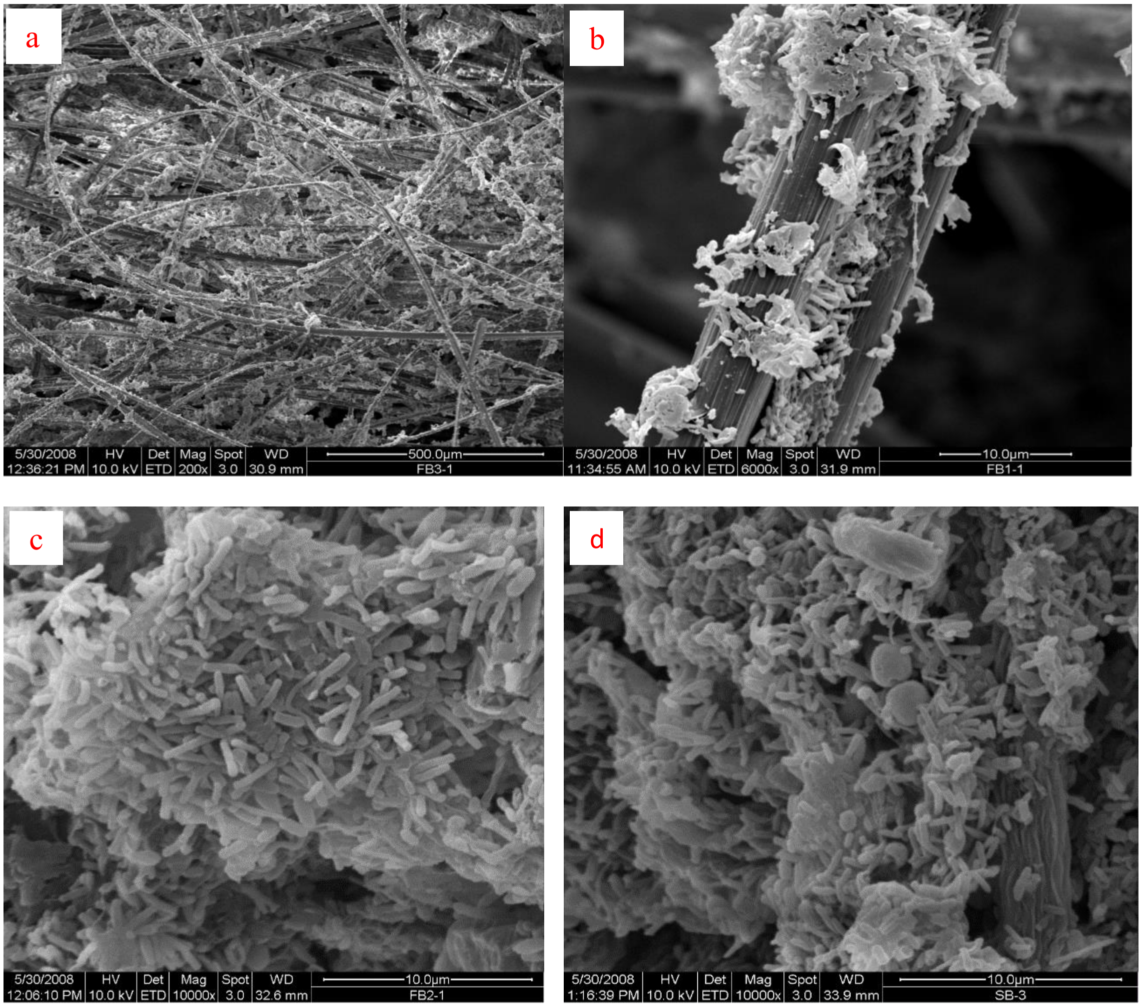

Fig. 29. SEM images of biofilm on flexible fibre with different magnifications of (a) 200x (b) 6000X (c and d) 10000x (Adapted from [78]).

5.2.7 Others

The analytical section "Others" collectively gathers materials which are not explicitly possible for insertion in a particular group. It consists of several articles reviewing various content sources.

5.2.7.1 Vegetal carbon (VC)

Similar to SP as earlier discussed in section 5.1.5.4, VC is not rated fit support for application in anaerobic digestion, since it allow for the appendage of unwanted organisms that struggle with methanogenic organisms for acetate substrate [82].

5.2.7.2 Rope-type media

In an FbSBR, high cover and filamentous-type of BC^{PLUS} media has the influence of preserving a high quantity of biomass on its matrix rapidly (≈ 7.7 g TS/m^2 media surface) with simultaneous nitrogen and organic removal. According to the authors [86], microorganisms were just found on the mold of media as biocatalyst in the reactor through the perpetual running phase.

5.2.7.3 Nylon nets

When NYLON nets were used as biocarriers, suspended and fixed biomass was made up of 20% NOB and 50% AOB. Optical microscopy of the reactor content showed the presence of metazoans and protozoans, suggesting a balanced treatment condition. A thick biofilm was found to be the fixed biomass and large numbers of *Rotatoria sp*, *Rotifers* and *Zooglea sp* were seen to be included [87]. Based on the aforementioned facts, it is highly suggested for utilization as a biocarrier material.

5.2.7.4 Eucalyptus charcoal

Methanogenic action appears to become the deciding factor to explain the greater effectiveness of the reactor packed with charcoal for organic matter extraction. At a COD/SO_4^{2-} ratio of 0.4, when the reactor was loaded with charcoal, only incomplete-oxidizing SRB metabolized basic organic matter with acetate, which is only transformed by sulfidogenic bacteria. This is the sole experimental situation under which SRB operation was the exclusive and dominant mechanism [79].

5.2.7.5 Porous polymer

Porous polymers as biocarriers are fit for microorganism's immobilization. After the start-up of SBBR [52], biofilm was seen to have successfully covered the biocarrier. Bacteria in the outside layer of the biofilm were principally aerobic species. Organic matter adsorbed to the biofilm acted as electron donators. Aeration loop positioned in the intermediate cylinder of the novel SBBR, provided sufficient DO. Thus, nitrification governed the outside layer of the biofilm in this cylinder.

5.2.7.6 Aluminum foil

Study by [88], showed that speedy initiation of SNAP and anammox process in biofilm reactor using aluminum foil without anammox sludge being inoculated is attainable. In their research, not only was biofilm produced to retain biomass and enrich AnAOB, tremendous parameters were properly monitored to suppress NOB proliferation, which helped establish an appropriate condition for AnAOB development, resulting to SNAP process rapid initiation. The microbial analysis inferred that microbial abundance of biomass improved while the microbial diversity decreased. *Candidatus Brocadia* and *Nitrosomonas* were enriched as the main AnAOB and AOB, respectively. *Nitrospira* was the main NOB and its relative bounty was reduced.

5.2.8 Composite packing

5.2.8.1 Composite of PE and inorganics (WD-F10-4 BioM^{TM})

The self-floating biocarrier (WD-F10-4 BioM^{TM}) was competent to develop active biofilm in the system operated by [65]. The biomass concentration quantified at the completion of the experiment amount to 1,301 mg/L of biomass appended on the biocarriers and 3,721 mg/L for the suspended biomass, indicating that the biofilm has higher activity than sludge.

5.2.8.2 GAC and natural zeolite

The effect of adding composite biocarriers (GAC and Natural zeolite) were studied. Even though, GAC and zeolite utilized in the research were alike in particle size, density and SSA. SEM photographs unveiled that, the GAC was extra permeable, had a buffeting surface with non-uniform structure than zeolite (Fig. 30). This could be the major reason why higher quantity of microbes were fastened to GAC than zeolite. Microbes willingly appended more to the fissures and uneven surfaces of the GAC biocarrier. However, the positive charge on zeolite aided the adherence of negatively charged microbes on to the covering of zeolite. Elongated rod shaped cells were fixed on to the exterior of both GAC and zeolite [71].

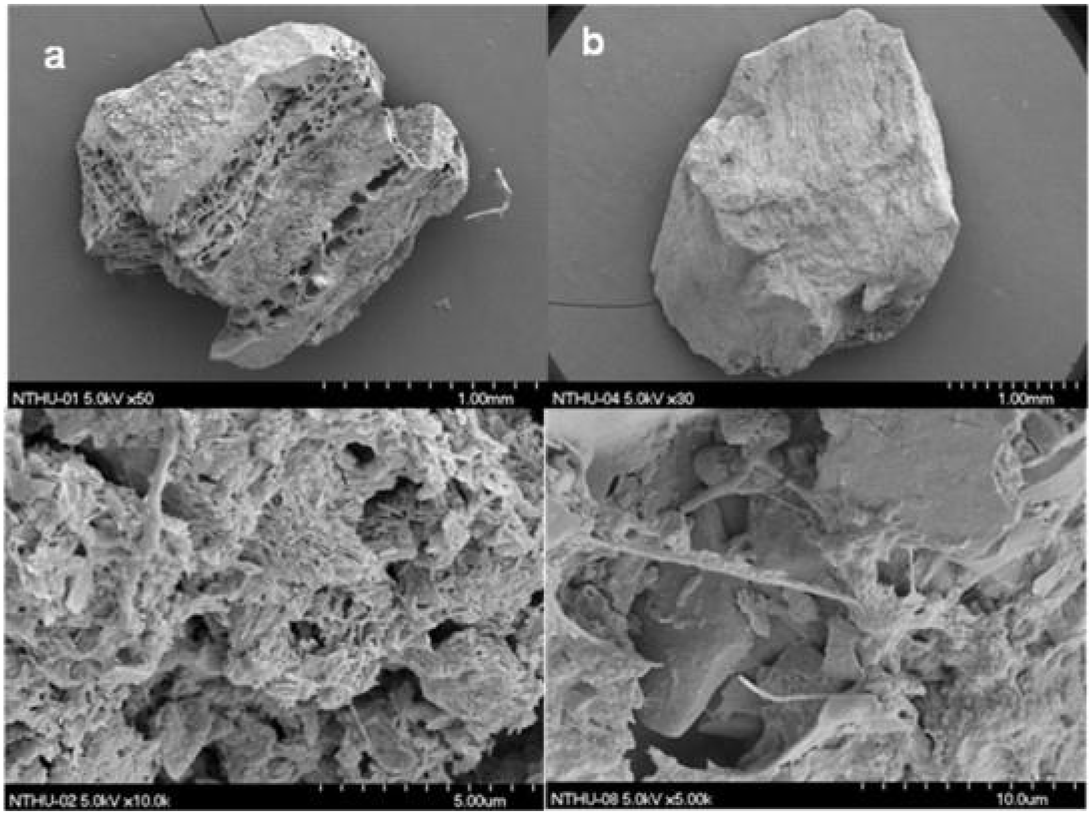

Fig 30. SEM photograph of (a) GAC external surface (top) and within (bottom) and (b) zeolite external surface (top) and within (bottom) (Adapted from [71]).

5.2.8.3 Floating spherical carriers (FSC)

The Floating spherical carriers (FSC) composite carrier created fluid isolation and was able to retrieve suspended matter that led to the liberation of dissolved organic matter by biofilm generation and biofilm lysis. The energy-uncoupling metabolism, slow-growth enrichment, sludge decay, hydrolytic and fermentation bacteria all led to the decline of in situ sludge in the floating spherical carriers-sequencing batch biofilm reactor (FSC-SBBRR). The FSC-SBBR generated 0.16 g MLSS/g COD, nearly 27.27% less sludge [85]. *Azospira* and *Mizugakiibacter* anaerobic effervescence bacterium were found to govern the FSC organisms, symbolising sludge decay as the principal mechanism on biofilm. Going by these findings, it can thus, be concluded that floating spherical carriers can adequately block swung sludge, enhancing biofilm production capability significantly.

5.2.8.4 Activated carbon fiber filler

SEM results indicates that a thick biofilm coated the filler surface, creating an anaerobic–aerobic microenvironment that facilitated nutrient removal. Dense biological membranes were also formed on the exterior of carbon fiber substances [76]. The SEM photographs of the biofilm further designate that the adhesion force of the interface is high with a real elastic force. Fig. 31B presents substantial amounts of cells in the structure of biofilms, sludge, and flocs. Fig. 31C and D, reveals that the exterior of the filler has a complicated structure mirroring the EPS molecules, which is liable for that in Fig. 31B. While Fig. 31D, presented a vast quantity of cocci, Bacillus, multi-streptococci and filamentous bacteria embedded in EPS framework, developing a thick biological network. This clearly indicates that, the activated carbon fiber filler demonstrated strong output in biofilm formation and microorganism immobilization. The major two parameters affecting biofilm growth are substrate loading rates and hydrodynamic conditions [25]. Consequently, various processes, including abrasion, erosion, sloughing and predator grazing, are said to be responsible for biofilm detachment.

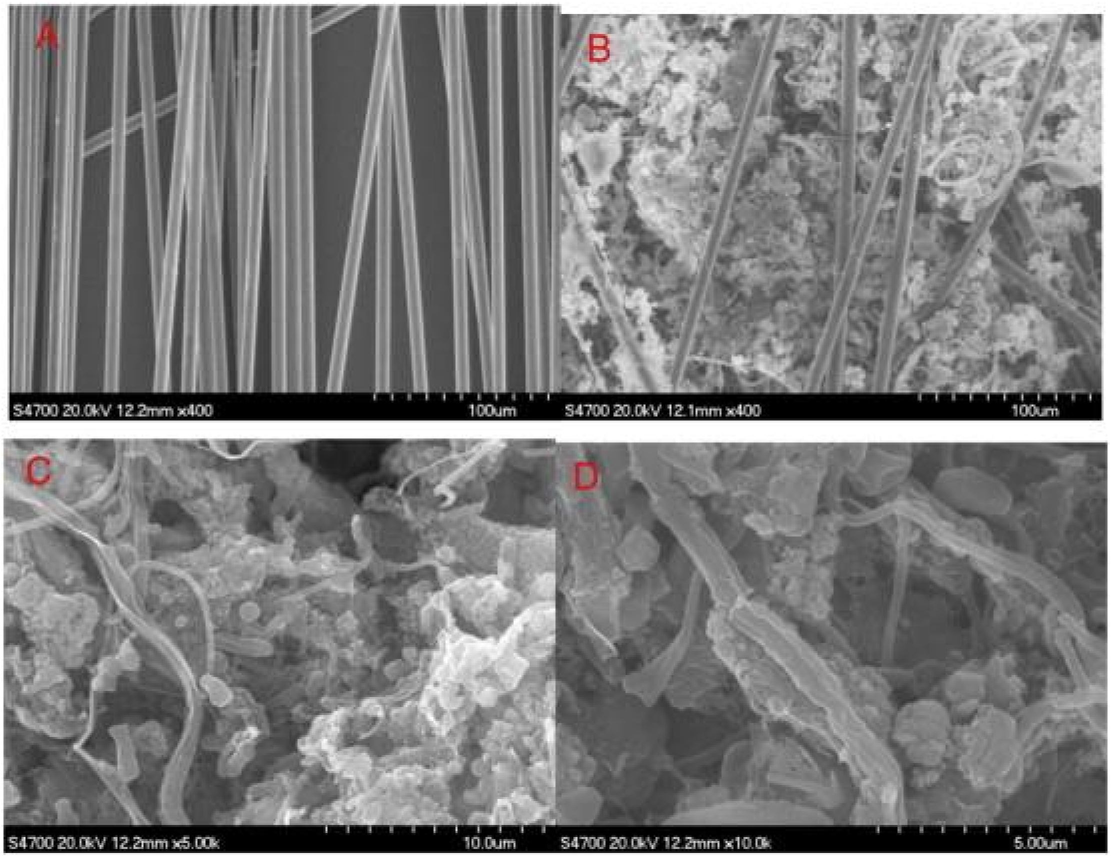

Fig. 31. SEM images of activated carbon fiber filler (A) and biofilm formed on the filler (B, C, and D) (Adapted from [76]

6.0 Conclusion

Numerous materials have been utilized as biocarriers in SBBR systems for simultaneous organic matter removal and biofilm formation during wastewater treatment, depending upon their availability and other specifications. Thus, showing different degrees of effectiveness. However, their extensive implementation is hindered by material property and relatively high operational and maintenance costs. This leads to the search for sustainable, environmentally friendly and economical compact biocarriers, which could retain sufficient treatment efficiency. Thus, this article presented a systematic review of existing research articles to advance our understanding of the properties and performance of various biocarriers with specific focus on their contributions in nutrient removal and biofilm formation. It further identified and discussed the limitations of these biocarriers, made comparative analyses and recommendations for better understanding.

The study, however, note the following:

- Biocompatibility with the predominant microorganism is very vital in selecting a biocarrier material.
- Waste materials offer a cost-effective, environment-friendly, and sustainable alternative as biocarriers for application in SBBR systems.
- Given the inherent disadvantages of synthetic biocarriers, it is recommended that natural materials with an additional incentive of reduced costs be used as an alternative biocarrier materials in SBBR for wastewater treatment.
- The advantage PE biocarriers have over other biocarrier materials is that biofilm can be distributed between its internal and external surfaces. Interestingly, PE can be pretreated to improve its physical properties. However, it does not encourage the predominance of any type of microorganism. Thermodynamic characterization indicated that PE had more favorable surface properties for the initial adhesion of biomass than PP. LS forms biofilm faster than most thermoplastic biocarriers.
- When PU foam was compared with some other biocarriers, it presented the fastest start-up and yielded constant effluent COD samples.

- SBBR systems developed with fibrous materials as biocarriers are highly efficient and energy saving wastewater treatment technologies.
- Composite packing: could occupy less space, run normally, operate in a stable manner, resist impact load, deal with higher hydraulic load and achieve better purification.
- PP biocarriers possess a longer life span than other thermoplastic materials providing better attachment than classical plastic biocarriers. Similarly, LS forms biofilm faster than most thermoplastic biocarriers while iron shavings are not suitable for biofilm formation

7.0 Future directions

In the recent years, biofilm technology is increasingly becoming largely employed for wastewater treatment under various loading and operational conditions, for effective organic and nutrient removal. Therefore, continued efforts are required in developing suitable biocarriers to address biofilm control issues. Researchers continue to concentrate on the improvement of biofilm technologies with special focus on biocarrier material. However, design and implementation of these technologies are hindered by the need for more appropriate biocarriers to account for the complexities of the heterogeneous communities that support substrates degradation. Hence, the following are suggested:

i. **Developing new and/or modification of existing biocarriers**: In any functional biocarrier, biofilm bioactivity must be significantly higher than suspended biomass. Therefore, searching for a novel biocarrier that would give rise to higher biofilm activity is important. While searching for the new biocarriers, researchers may consider trying the following: (i) modifying the existing ones could also be an option such as, doping fibers on existing biocarrier materials or specially manufactured membranes for application in SBBR. (ii) application of plastic waste or fibers extracted from cellulose of by-products of major agro-based materials as biocarriers. This will also go a long way towards utilizing waste.

ii. **Composite biocarriers:** Biocarriers despite their demonstrated effectiveness in treating wastewaters, have their own limitations and are yet to be investigated for large-scale applications. Therefore, future efforts may be focused on improving such biocarriers to reduce space and energy consumption as well as gas and odor emissions. Future researchers should further investigate the efficiency of composite biocarriers instead of stand-alone biocarriers. A thorough understanding of stand-alone biocarriers and its modification as a composite has a vast potential to be the future trend for use in wastewater treatment. More studies are required to be conducted in making composite biocarriers e.g. coating Banana trunk fiber with chitosan composite after durability and surface integrity test have been investigated.

iii. **Biocarrier coating:** Commercial plastic media has limited surface area, and this has limitation to its use when high biofilm growth rate is required. The development of activated carbon on the surface of plastic biocarriers for biofilm system in order to provide a large surface area for microbes to attach would result in reduced retention time. Hence, making the treatment plant more compact and effective. It also retains suspended microorganisms within the bioreactor, thereby significantly reducing plant footprint and produces less sludge. More so, knowing fully that nanoporous carbons (NPCs) possesses highly porous structure and enlarged surface area, their utilization combined with plastic biocarriers would be remarkably valuable in adsorption and separation processes for pollutant degradation because of its high adsorptive performance. According to findings of this review, only GAC re-generated from coconut shells/husk has been used as a biocarrier in SBBR. Therefore, future researchers should conduct more studies using biomass materials like apricot shells, walnut shells, bamboo etc.

iv. **3D printing:** The optimization of biocarriers application in SBBR systems in order to increase efficiency and reduce operating costs has not been deeply explored. Therefore, more research should be done to vehemently optimize various biocarrier materials for application in SBBR systems during the treatment of various wastewater types. During this review, it has been observed that most researchers focuses on the optimization of the environmental and operational parameters such as Temperature, salinity, HRT, SRT, cycle time etc, thus, forgetting to optimize biocarrier properties such as density, thickness, specific area, size, shapes, etc. The 3D printing technique using most recent and sophisticated machines would significantly help in optimizing the aforementioned biocarrier properties at minimal cost and in less time.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Abbreviation

AS:	Activated Sludge
AOB:	Ammonia Oxidizing Bacteria
AOX:	Adsorbable Organic Halides
APSBBR:	Alternating Pumped Sequencing Batch Biofilm Reactor
ASBBR :	Anaerobic Sequencing Batch Biofilm Reactor
CANON:	Completely Autotrophic Nitrogen Removal Over Nitrite
COD:	Chemical Oxygen Demand
DEAMOX:	Denitrifying Ammonium Oxidation
DNPAOs:	Denitrifying Phosphate Accumulating Organisms
EPS:	Extracellular polymeric substances
FB-SBBR:	Fixed-Bed Sequencing Batch Biofilm Reactor
FSBR:	Fixed-Bed Sequencing Batch Reactor
FSC-SBBR:	Floating Spherical Carriers-Sequencing Batch Biofilm Reactor
FbSBR:	Fixed-bed Biofilm Sequencing Batch Reactor
HG-SBR:	Hybrid-Growth Sequencing Batch Reactor
HRT:	Hydraulic Retention Time
HSBR:	Hybrid Sequencing Batch Reactor
IFAS-SBR:	Integrated fixed-film Activated Sludge-Sequencing Batch Reactor
GAC:	Granular Activated Carbon
GAC-SBBR:	Granular Activated Carbon Sequencing Batch Biofilm Reactor
LS:	Luffa Sponge
MB-aerobic-SBR:	Moving biofilm aerobic Sequencing Batch Reactor
MBBSBR:	Moving-Bed Biofilm Sequencing Batch Reactor
MBSBBR:	Moving Bed Sequencing Batch Biofilm Reactor
MC:	Mineral Coal
MCRT:	Mean Cellular Retention Times
MLSS:	Mixed Liquor Suspended Solid
MLVSS:	Mixed Liquor Volatile Suspended Solid
MM-SBBR:	Multimedia-Sequencing Batch Biofilm Reactor
MSBR:	Sequencing Batch Reactor Biofilm
NLR:	Nitrogen Loading Rate
NOB:	Nitrite Oxidizing Bacteria
NSBBR:	Natural Zeolite Sequencing Batch Biofilm Reactor
PAOs:	Phosphate Accumulating Organisms
POC:	Palm Oil Clinker
PN:	Partial Nitrification
PU:	Polyurethane
PE:	Polyethylene
PEHD:	Polyethylene high-density
PP:	Polypropylene
PVC:	Polyvinyl Chloride
SBR:	Sequencing Batch Reactor
SBBR:	Sequencing Batch Biofilm Reactor
SBBR-VFCW:	Sequencing Batch Biofilm Reactor combined with a Vertical Flow Constructed Wetland
SBCR:	GAC-Biofilm Configured Sequencing Batch Reactor
SBFFBR:	Sequencing Batch Flexible Fibre Biofilm Reactor
SBMBfR:	Sequencing Batch Membrane Biofilm Reactor
SBGR:	GAC-Biofilm Sequencing Batch Reactor

SBMBBR: Sequencing Batch Moving Bed Biofilm Reactor
SBMABR: Sequencing Batch Membrane-Aerated Biofilm Reactor
SBR-BF: Sequencing Batch Reactor-Biofilm
SC-SBBR: Suspended Carriers-Sequencing Batch Biofilm Reactor
SEM: Scanning Electron Microscope
SLR: Systematic Literature Review
SNAD: Simultaneous Nitrification and Denitrification
SNDPR: Simultaneous Nitrification and Denitrification and Phosphorous Removal
SRB: Sulphate-Reducing Bacteria
SRT: Solid Retention Time
SSA: Specific Surface Area
SS: Suspended Solids
SSBBR: Synthetic Zeolite Sequencing Batch Biofilm Reactor
SP: Synthetic Pumice
TN: Total Nitrogen
TP: Total Phosphorus
TVS: Total Volatile Solids
WAS: Waste Activated Sludge
VC: Vegetal Carbon

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