Vol. 13 No. 1 (2020)

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ISSN 1820-7480

RECYCLING & SUSTAINABLE DEVELOPMENT

RECIKLAŽA I ODRŽIVI RAZVOJ

Editor-in-Chief:

MILAN TRUMIĆ

Publisher:
Technical Faculty in Bor, University of Belgrade, Serbia

RECYCLING & SUSTAINABLE DEVELOPMENT

Journal info:

The Journal is issued one time a year, indexed and abstracted in Chemical Abstracts and SCIndeks, DOAJ digitally archived at the National Library of Serbia and in the Repository of SCIndeks - The Serbian Citation Index as the primary full text database.

ISSN 1820-7480 Print; ISSN 2560-3132 Online
www.rsd.tfbor.bg.ac.rs
rsdjournal@tfbor.bg.ac.rs

Publisher: Technical Faculty in Bor, University of Belgrade, Serbia
Print: Grafomed trade Bor, Serbia

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www.rsd.tfbor.bg.ac.rs

Journal Recycling & Sustainable Development
is financially supported by the Ministry of Education,
Science and Technological Development, Republic of Serbia

Recycling and Sustainable Development

www.rsd.tfbor.bg.ac.rs

RSD

Online ISSN 2560-3132
Print ISSN 1820-7480

Assessment of composting processes in an automated aerobic fermentation system based on key parameters

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ARTICLE INFO

Received 08 June 2020
Accepted 15 September 2020

Research article

Keywords:
Chicken manure
Fermentation
Composting
Temperature and moisture distribution model
Key parameters

ABSTRACT

The poultry (broiler) industry is continuously increasing all over the world, therefore, the amount of waste generated in these facilities is also increasing. As a consequence of the above mentioned, the more efficient conversion of chicken manure to organic fertilizer is a key problem. The aims of the study are to investigate the changes in temperature and moisture content during composting of chicken manure in the oval tank fermentation system and to create a model for the evaluation of the performance of biodegradation processes. The moisture and temperature distribution models of the oval tank were created in Hydrus software. The results showed that the oval tank fermenter can be divided into two main zones. In the first zone, where the rate of biodegradation was relatively high, a heterogeneous temperature zone was found with continuously decreasing moisture content. The second zone was more homogenous in both temperature and moisture content. This stage represents the weak fermentation part of the technology and results in an elongated post fermentation section. Furthermore, statistically significant correlations were found between composting key parameters, such as ammonium content with temperature and organic matter content with organic nitrogen content. It was also concluded that the exact location of the manure turning and chopping mechanical system (MTCM) used for aeration had a high effect on the composting processes, as well as on the quality parameters of the mature compost.

1. Introduction

Composting is a well-known method in waste management that can be applied to the treatment of biodegradable wastes. The primary aim of composting is to decrease the effects of hazardous potential on the environment, as well as the volume of the generated organic wastes. It is an exothermic, aerobic degradation process where the final product is a stable, valuable material (equation 1) which can be used for different purposes (e.g. soil amendment) (Ashraf and Gregg, 2020).

$$\textit{organic waste} + O_2 \xrightarrow{\textit{microorganisms}} CO_2 + H_2O + \textit{stable compost} + \textit{heat} \qquad (1)$$

In the industrial scale fermentation facilities, the quality of the mature compost can be controlled by setting up the initial parameters of the feeding material and the operational circumstances. There are some key

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doi: 10.5937/ror2001001M

parameters, which should be considered during the degradation process, since they have a significant effect on the final product quality (Asadu et al., 2019). One of the most important is the initial moisture content of the feeding material, because the aqueous medium is essential for the metabolism of microorganisms and for the enzymatic catalytic reactions. The optimal moisture content is in the range of 40 - 60 V/V % (Madejón et al., 2002). The higher moisture content inhibits the degradation process and turns it to an anaerobic degradation.

The decomposition process can be tracked by monitoring the temperature (Fernandes et al., 1994). The temperature provides information about which phase the degradation is in (Figure 1).

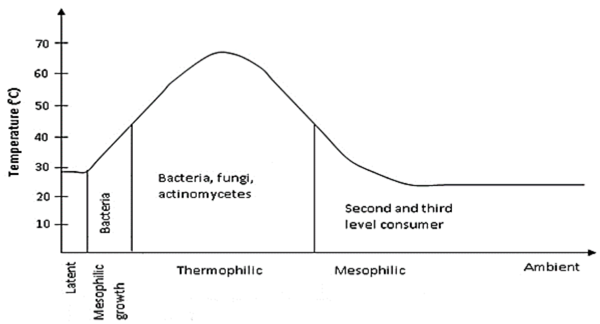

Figure 1. Patterns of temperature and microbial growth during composting (Bhatia et al., 2015)

According to Figure 1, a temperature elevation can be observed within a few hours after filling in the material to the fermentation system. The maximum temperature arises in the thermophilic phase and thereafter it starts to slightly decrease in the mesophilic phase until reaching the ambient temperature.

The carbon/nitrogen ratio is also a key parameter from the aspect of microorganisms. The microorganisms use these chemical elements as a cell-building substance (carbon for energy and nitrogen for protein production). The optimal C:N ratio should be in the range of 25 - 30:1 (Asadu et al., 2019). This ratio can be adjusted to the optimal by using additive materials (e.g. saw dust or straw).

The chicken manure is generated in large amounts and relatively rich in nitrogen, as well as in potassium and phosphorus, therefore, it is one of the most often used materials for composting (Amanullah et al., 2010; Szabó et al., 2019). However, the chicken manure as waste from the poultry industry includes a mixture of excreta (manure, faeces, and urine), bedding material or litter (e.g. wood shavings or straw), waste feed, carcasses, broken eggs, and feathers removed from poultry houses (Raviv et al., 1999). Other wastes include those from cage, conveyer belt and water-flushing systems. Poultry manure is acquired through the regular cleaning of the poultry house (Kobierski et al., 2017).

The litter and manure component of the poultry house waste have a high nutritional value, and consequently they can be used as an organic fertiliser, thus recycling the nutrients such as nitrogen, phosphorous, and potassium. The poultry litter has traditionally been spread on soil as an amendment (Rynk et al., 1991). The mature compost can improve soil fertility and plant growth (Haga, 1999), however, immature compost applied on soil can contribute to N starvation (Bernal et al., 2009; Moral et al., 2009), phytotoxic effects, and presence of harmful microbes (Fang et al., 1999; Tiquia and Tam, 2000).

The high nitrogen content and balanced nutrients are the reason that chicken manure compost is the best kind of manure to use. It should be considered that the high nitrogen in the chicken manure can be dangerous to plants if the manure has not been properly composted. Raw chicken manure fertilizer can burn or even kill plants. Moreover, over-application of this material can lead to an enriching of water nutrients resulting in eutrophication of water bodies, the spread of pathogens, the production of phytotoxic substances, air pollution, and emission of greenhouse gases (Fan et al., 2000; Kelleher et al., 2002). Bitzer and Sims (1988) reported that excessive application of poultry litter in cropping systems can result of nitrate contamination in groundwater. Excessive application of fresh poultry manure on the farm may result in an excess accumulation of ammonia and damage the crop roots too (Köteles and Pereş, 2017; Tamás et al., 2017). Proper handling of the manure can be achieved through proper engineering of composting and appropriate practices of feed management (Bolan et al., 2010).

2. Materials and methods

2.1. Feeding material

Deep litter chicken manure and dehydrated hen manure were used from the Kisvárda slaughterhouse as a feeding material in the fermentation procedure. The maximum capacity of the plant is 11,000 t/year, which represents a 30 t daily usage. Taking this into account with the average composition of boiler chicken manure, a large amount of nutrient content (330 t of N, 220 t of P_2O_5 and K_2O) can be theoretically recovered and used as fertilizer.

The mixing ratio of the raw materials in the fermentation tank was 1/3 of broiler and 2/3 of chicken manure. The moisture content of the incoming chicken manure varied between 20 and 40 %. For the optimal fermentation the moisture content of the raw material should be adjusted to 40 - 45 % by adding water (150 l/intake).

2.2. The automated fermentation system

The Hosoya (oval tank) fermentation and drying technology is a Japanese-developed alternative method
technology is a Japanese-developed alternative method

for poultry and pig manure. The Hosoya technology was launched in 1970 and is still being developed.

A study was undertaken by Georgakakis and Krintas (2000) to investigate and optimize an oval tank composting system in composting poultry manure at a typical poultry farm in Greece. The oval tank fermentation system is one of the two different treatment systems that have been applied for the treatment of wastes. The other one is the so-called Okada system, both of them of Japanese origin. These systems are based on the operation of specially designed manure turning and chopping mechanical systems (MTCM). The system consists of a series of rotating metallic knives or forks with which the waste is completely turned, aerated, and gradually pushed to the end of the specific tank. This installation consists of an open, shallow, and oval shaped concrete tank (Figure 2). The standard size of the tank is 60 m × 8.3 m.

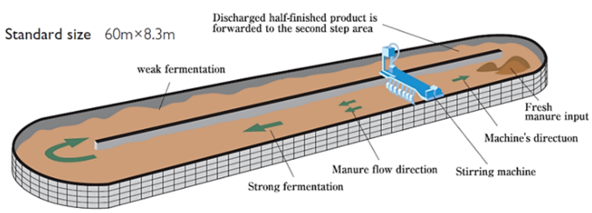

Figure 2. Arrangement and processes of the automated industrial fermentation system

Fresh manure is batch fed daily to the oval tank and an equivalent quantity of final material is removed from the exit. The tank is filled with the waste material up to a total depth of 1.0 - 1.2 m. The stirring machine with double rotors ensures the continuous mixing of the manure in the fermentation tank.

The MTCM in the fermentation system completes a full run along the oval tank in approx. 2.5 h. On a daily basis, a maximum of 5 full runs can then be completed. One complete run results in the displacement of 1.5 m of manure along the tank or a maximum of 7.5 - 8.0 m after 5 runs completed in 24 h. Therefore, the minimum travelling time for fresh manure to reach the exit of the 120 m long channel is 12 - 14 days. During the turning and pushing of manure by the MTCM system, surrounding air is incorporated and moisture is lost by evaporation. The fermentation system controls the initial moisture content by mixing the incoming fresh manure with the recirculated dry old material in the channel and this helps to start the composting process. Moisture control of the material in the oval tank is necessary to avoid blockages of the MTCM operation (Hosoya and Co., 1996). A particle size of less than approx. 12 mm is formed from the initially muddy-textured raw material due to the turning effect of the MTCM system in the oval channel. The reduction of particle size accelerates the degradation due to the higher specific surface area available for microbes and increases the porosity providing the appropriate aeration conditions. In the technology, 3 - 5 cm thick fermented manure remains at the bottom of the fermentation tank as a microbial starter.

In this paper the performance of the oval tank fermentation system was studied by taking samples of poultry manure during a run and analyzing them for moisture content, dry matter content, and different nitrogen forms, such as organic nitrogen and inorganic forms (nitrate and ammonium). The temperature of the material during the fermentation was also monitored.

2.3. Sampling strategy

In order to create an appropriate model for the temperature and moisture distribution of the oval tank, temperature measurements and sample collections were performed monthly, from April to November in 2019. In this paper, the results of the June measurements represent the effect of hot and dry circumstances on the processes of the fermentation. Sampling cross sections was adjusted to the sensors of the fermentation tank based on earlier observations (Figure 3). In total, 21 sampling cross sections were selected and placed more frequently after the feeding point because of the initial strong fermentation, where higher temperature and moisture gradients were expected.

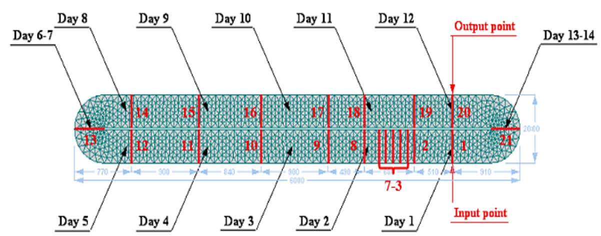

Figure 3. The location of sampling cross sections (days show the movement of the manure)

The temperature of the material was measured at three different points along each of the cross sections. One was the middle of the tank and the other two measuring points were 30 cm from the edges of the tank. Every measuring point was on the bottom of the tank. The samples were collected from the middle of each section for analytical purposes.

2.4. Applied measuring methods

Testo 922 mobile thermometer was used for temperature measuring (accuracy ± 0.5 °C). The moisture content was determined from the collected samples by weight loss method in the laboratory (MSZ-08-0221-1:1979). The moisture content was measured by drying in an oven at 105 °C for 12 h, or until constant dry weight was obtained.

In order to determine the exact quantity of inorganic nitrogen forms the Kjeldahl method was used alongside the photospectrometric method in accordance with the Hungarian Standards (MSZ-08-1744-1:1988; MSZ 20135:1999). The quantity of organic nitrogen was calculated based on the previously described methods. The organic content was estimated by the loss on ignition method (MSZ-08-0012-6:1981).

2.5. Modelling software

The two-dimensional version of the Hydrus software was used for modelling the water and temperature distribution in the oval tank during the biodegradation processes. The software applies a finite element model for simulating the movement of water, heat and multiple solutes in variably saturated media. The mathematical background of the software was the Richards equation for saturated-unsaturated water flow and convection-dispersion type equations for heat and solute transport.

The 2D geometry of the fertilizer oval tank was created as a first step, where the target finite element size was adjusted to 0.1 m. The duration of biodegradation processes was set to 14 days in the model according to the fermentation technology. The applied main parameters of feed material were 800 kg/m^3 bulk density, 55 % solid content, and 70 % organic content in the solid matter. In addition, the previously carried out results of in situ water content and temperature measurements were used as initial conditions. Finally, the “no flux” boundary condition was applied at the inner and outer circumference of the model tank.

3. Results and discussion

In this chapter, the results of temperature and moisture distribution models, as well as the analytical evaluation was introduced in June 2019. The correlations among the investigated parameters were statistically performed to obtain a comprehensive view about how the different parameters can effect on the performance of the fermentation system.

3.1. Temperature and moisture distribution models

The highest moisture content (expressed in grams/unit mass) was measured at the feeding point (0.428) (Figure 4), however, this value was adjusted for optimization at the beginning of the biodegradation processes and remained constant until the sampling point 9 (16.8 m). A rapid and sudden decrease can be observed after the high moisture content section as a result of the intensive heat generation in the thermophilic phase (between sampling point 9 and 13). The other part of the oval tank (after turning point) showed a slightly decreasing moisture content. The moisture content of the output material represented approximately 0.204, which was appropriate for pellet production from the composted material by pressure agglomeration technologies.

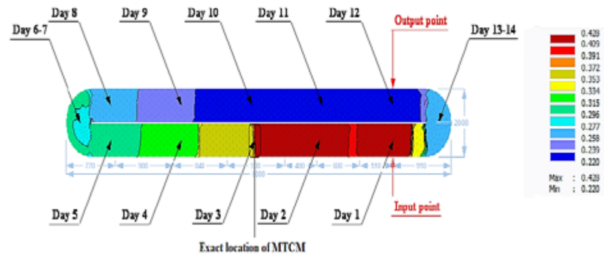

Figure 4. The moisture content model of the fermentation system (June 2019)

The fermentation tank can be divided into two main zones including the decomposition phases based on the results of the temperature distribution model (Figure 5). Heterogeneous temperatures were measured between sampling points 1 and 12 (this strong fermentation zone was divided into mesophilic and thermophilic phases by the MTCM system), as a result the inner, centre, and outer parts of the oval tank could be separated from each other. The centre part had the highest average temperature value (50.55 °C), higher on average than 7.27 °C compared to the inner parts and 3 °C compared to the outer parts. Weak fermentation zone (transient and maturation phases) was observed from the turning point (sampling point 13) to the output point (sampling point 21). The average temperature in this zone was 33.42 °C, the calculated standard deviation was ± 1.54 °C. The ambient temperature was 24.4 °C, when the measurements were carried out.

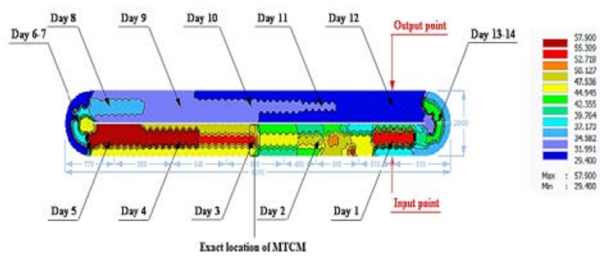

Figure 5. The temperature distribution model of the fermentation system (June 2019)

The temperature heterogeneity in the fermentation tank can be explained by the exact location of MTCM system. According to the above mentioned, higher microbiological activity and heat generation were formed, where the MTCM passed through earlier.

3.2. Analytical results

The correlation between temperature and ammonium is shown in Figure 6. The maximum measured temperature was 57.9 °C in the thermophilic phase, where a local minimum of ammonium concentration was also observed due to the exact location of the stirring machine. The MTCM intensified the ammonium release from the compost material to the environment during the aeration.

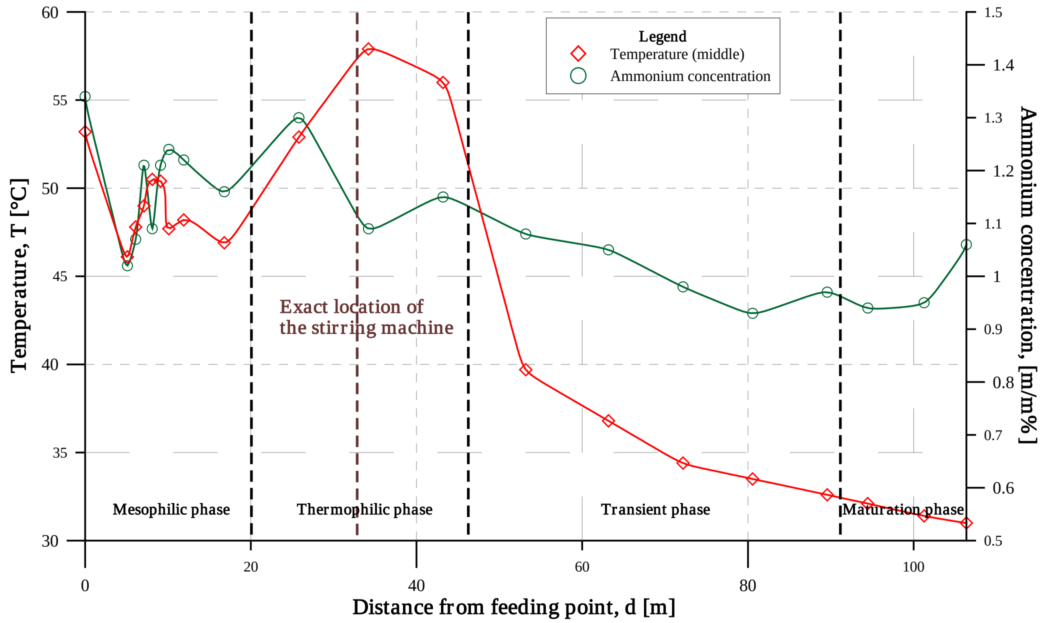

Figure 6. The measured temperatures and ammonium concentrations in the fermentation system (June 2019)

The mesophilic phase lasted for approximately 20 m in the oval channel, followed by the thermophilic phase (26 m), and a relatively long transient phase (46 m) according to the temperature measurements. At the end of the fermentation process (maturation phase) the initial ammonium concentration (1.34 m/m %) decreased by 29.1%. The significant correlation between the two parameters was also statistically proved (R^2=0.734; $p<0.05$).

The results of organic content and organic nitrogen content measurements can be seen in Figure 7. The organic content was in the range of 68.6 - 82.0 m/m %, while the organic nitrogen content represented 3.2 - 5.43 m/m % in the oval tank. In case of both parameters, the peak values were found near to the input point (sampling cross section 5). After reaching the maximum values, a rapid decrease can be seen on the curves in the following 35 meters. A strong linear correlation was found between organic content and organic nitrogen content (R^2=0.841; $p<0.05$).

The rates of different nitrogen forms were also examined during the composting processes (Figure 8). The predominance of organic nitrogen (73.32 - 83.15 %) was clearly observed in contrast to ammonium (16.74 - 26.4 %) within the nitrogen forms. The rate of nitrate was negligible and it varied in the range of 0.11 - 0.28 %.

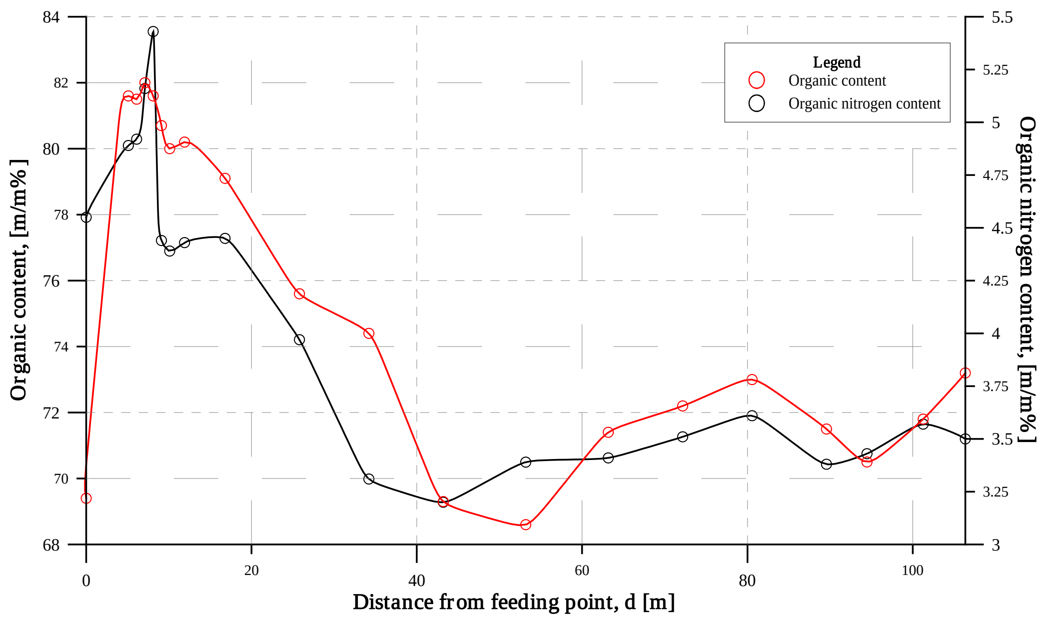

Figure 7. The measured organic contents and organic nitrogen contents in the fermentation system (June 2019)

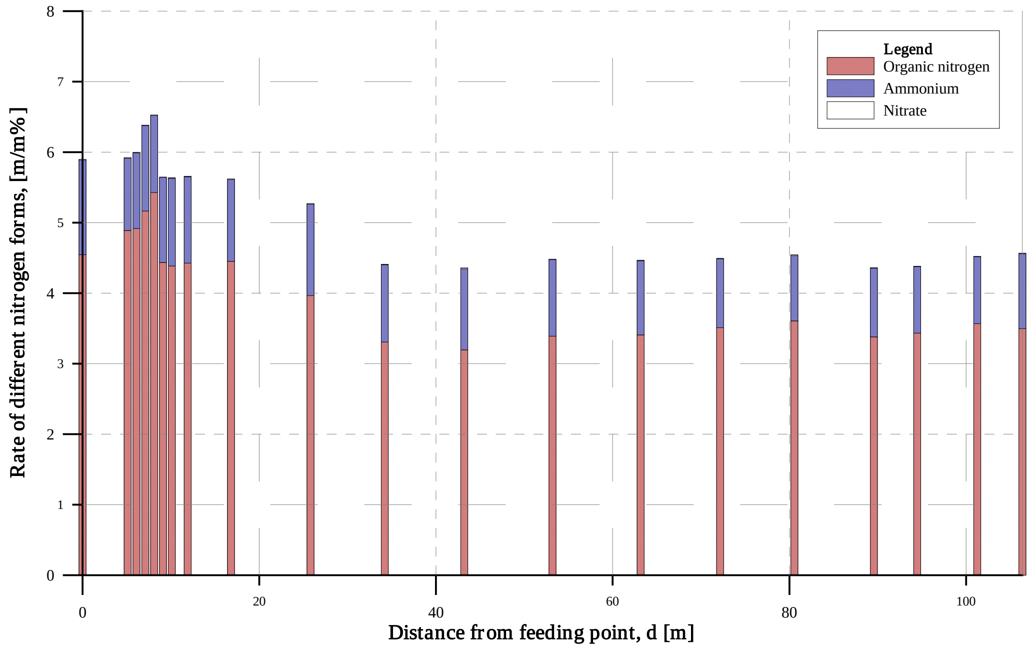

Figure 8. The rates of different nitrogen forms in the fermentation system (June 2019)

4. Conclusions

The performance of the oval tank fermentation system was studied by taking samples of poultry manure during a run and analyzing them for moisture content, dry matter content, and different nitrogen forms, such as organic nitrogen and inorganic forms. The temperature of the material during the fermentation was also monitored.

The two dimensional version of the Hydrus software was used for modelling the moisture and temperature distribution in the oval tank during the biodegradation processes. The results pointed out that the initial turning and mixing contributed to a high temperature and moisture content drop. It was found that the studied oval tank fermenter can be divided into two main zones considering the changing temperature and moisture content. In the first zone, where the rate of biodegradation was high, there was a heterogeneous temperature zone with continuously decreasing moisture content. The second zone was more homogenous in both temperature and moisture content. This stage represented the weak fermentation part of the technology and resulted in an elongated post fermentation section. The changes of temperature and moisture content along the tank fermenter were the same and there was a strong connection between them in the examined period.

Furthermore, statistically significant correlation was found between ammonium content and temperature as well as between organic matter content and organic nitrogen content. It was also concluded that the exact location of the MTCM system used for aeration has a high effect on the composting processes, thus on the quality parameters of the mature compost too.

The results pointed out that after the thermophilic phase there was sufficient time for composting. Therefore, this system can be used as an efficient treatment for chicken manure to decompose and obtain a valuable base organic fertilizer.

Acknowledgment

The research was carried out and financed by GINOP-2.2.1-15-2017-00043 project at the University of Debrecen.

The research was financed by the Thematic Excellence Programme of the Ministry for Innovation and Technology in Hungary (ED_18-1-2019-0028), within the framework of the Space Sciences thematic programme of the University of Debrecen.

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Procena procesa kompostiranja u automatizovanom sistemu aerobne fermentacije na osnovu ključnih parametara

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INFORMACIJE O RADU

Primljen 08 jun 2020
Prihvaćen 15 septembar 2020

Originalan rad

Ključne reči:
Živinski stajnjak
Fermentacija
Kompostiranje
Model za distribuciju vlage i temperature
Ključni parametri

I Z V O D

Živinarska industrija (uzgajanje brojlera) se kontinuirano razvija u celom svetu, a samim tim raste i količina otpada nastalog u tim postrojenjima. Kao posledica gore navedenog, efikasnije pretvaranje živinskog stajnjaka u organsko đubrivo predstavlja ključni problem. Cilj ovog istraživanja je ispitati promene temperature i sadržaja vlage tokom kompostiranja živinskog stajnjaka u ovalnom rezervoaru za fermentaciju i napraviti model za procenu efikasnosti postupka biorazgradnje. Modeli distribucije vlage i temperature u ovalnom rezervoaru su kreirani u softveru Hydrus. Rezultati su pokazali da se ovalni rezervoar za fermentaciju može podeliti u dve glavne zone. U prvoj zoni, gde je stopa biorazgradnje bila relativno visoka, utvrđeno je postojanje heterogene temperaturne zone sa konstantnim opadanjem sadržaja vlage. Druga zona je bila homogenija u pogledu temperature i sadržaja vlage. Ova faza predstavlja slabu fermentaciju u okviru tehnologije i kao rezultat toga dolazi do stvaranja izduženog područja nakon što se postupak fermentacije završi. Osim toga, utvrđene su i statistički značajne korelacije između ključnih parametara kompostiranja, kao što je korelacija između sadržaja amonijuma i temperature i između sadržaja organske materije i organskog azota. Takođe je zaključeno da tačna lokacija mehaničkog sistema za okretanje i usitnjavanje stajnjaka (MTCM), koji se koristio za aeraciju, ima veliki uticaj na proces kompostiranja kao i na parametre kvaliteta zrelog komposta.

Recycling and Sustainable Development 13 (2020) 9-21

www.rsd.tfbor.bg.ac.rs

Recycling and Sustainable Development

RSD

Online ISSN 2560-3132
Print ISSN 1820-7480

Determination of circularity indicators - a case study of MB International Company

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ARTICLE INFO

Received 30 June 2020
Accepted 25 September 2020

Research article

Keywords:
Circular economy
Circularity Indicators
Material Circularity Indicator
Sustainable development

Extended and revised version of a conference article - presented in 3rd ICQL

ABSTRACT

The circular economy (CE) is currently a worldwide popular concept that should ensure sustainable development and resource efficiency. It is established on the theory of consumption and use of resources in the process of production in a way that affects a limitation of adverse effects on the environment. Simultaneously, this concept creates additional value and reuse of the products. In the Republic of Serbia (RS), the idea of CE is still new and underdeveloped. Hence, this paper aims to explore the possibility of implementing a CE in companies that operate in the RS by adopting the already developed methodology in the European Union. This research was conducted by monitoring the production process in the company "MB INTERNACIONAL" that produced cardboard packaging. The obtained approximate value of Circular Indicator of this company was 0.47, which indicated that the company had excellent chances for full implementation of the CE model in the business with the application of specific measures. The low-budget and high-budget measures, which could improve the circularity level in the analyzed company, are also presented in the research.

1. Introduction

Found on the latest available United Nations report, the total population of the world exceeded 7.7 billion in 2019 (United Nations, 2019). The growth of the population results in problems and risks which endanger the environment and affect the inability to achieve sustainable development. In order to eliminate those problems or reduce them, it is necessary to make specific changes and modifications to the current economic model to improve the environment's quality.

The current global economic model entitled the linear economy model (LEM) is based on the "take-make/use-dispose" principles (Levoso et al., 2019).

There are many diverse negative outcomes from the take-make/use-dispose linear economy model (Andrews, 2015). The LEM consists of one linear flow of the materials that is not sustainable: the extraction of resources and their use in production, distribution of products, market placement, use of products by consumers, and finally, product disposal in the form of waste. Improvement of the said model can be achieved by different preventive measures that have defined the modern concept of economy named circular economy

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doi: 10.5937/ror2001009P

model - that has the potential to lead to sustainable development while decoupling economic growth from the negative consequences of resource depletion and environmental degradation (Morseletto, 2020).

The circular economy model represents the model which replaces the end-of-life concept with restoration, shifts towards the use of renewable energy, eliminates the use of toxic chemicals, which impair reuse and return to the biosphere, and aims for the elimination of waste through the superior design of materials, products, systems, and business models (EMAF, 2013). The transition from a linear to a circular economy represents a systemic change that builds long-term business and economic reforms and provides environmental and social benefits. Implementation of the CE is a very complex activity because it requires changes in the whole chain of product values: design and new business models, new ways of transforming waste into resources, new ways of consumer behavior, etc. The circular economy focuses on the efficient use of materials, reduction of waste and recycling of materials (Kirchherr et al., 2017).

The Republic of Serbia needs to align the national legislation with the European Union (EU), as a candidate for the EU membership. That implies harmonizing legislation in all fields, including the field of environmental protection. In the analyzed area, EU "Circular Economy Action Plan: For a cleaner and more competitive Europe" (European Commission, 2020) should be included in the Serbian policy. This plan has been built on the work done since 2015 when the first version of the action plan "Closing the loop - An EU action plan for the Circular Economy COM/2015/0614 final" was adopted (European Commission, 2015). The action plans for the CE launched by European Commission define concrete and ambitious programs, with measures covering the entire life cycle of a product, from production and consumption to waste management and the secondary raw material market.

According to this action plan, estimation of the level of circularity in companies should be executed. Today, in the Republic of Serbia, there is no tool available to assess the economic and environmental performance of circular systems. Consequently, the motivation for this research is represented by the fact that there are still no officially developed indicators related to the circular economy in the Republic of Serbia. For that purpose, the adoption of the existing "Circularity Indicators" was made. The methodology was developed by the EMF and Granta in the MCI project's context aiming to find indicators to measure how well a product performs in the CE system (EMAF, 2015).

2. Theoretical framework

The CE represents a contrast to today's model of the economy that includes the uncontrolled exploitation of natural material and its flow from the factory across consumer to the landfill (Đurić et al., 2017). An idea of the CE evolved from the fact that waste does not exist in nature. The cycles in nature (e.g. water cycles, nutrient cycles) exist to help the waste of one cycle become the resource of the other (Patwa et al., 2020).

Based on the question of why the concept of circular flows of natural materials was not applied in production processes, the CE began to evolve. A CE is characterized as an economy that is regenerative by design aimed to retain as much value as possible of products, parts, and materials. In other words, to create a system that allows for the long life, optimal reuse, refurbishment, remanufacturing, and recycling of products and materials (EMAF, 2016; Kraaijenhagen et al., 2016). The main goal of a circular economy is to protect the environment against any form of degradation and to improve the socio-economic conditions of a particular society (Solaja, 2019).

The development of CE cannot possibly be related to one single date or one individual researcher. Many scientists claim that Pearce and Turner were first to establish the concept of CE. They made the theoretical framework based on previous studies of the scientist Kenneth Boulding (Pearce and Turner, 1990), but they were not recognized as founders of the CE concept.

Germany was the first country to formally accept the CE concept and incorporate ideas of the concept into its legislation. The CE concept was defined in The law of closed substance cycle and The waste management act in 1996 (El-Haggar, 2007). Japan did the same thing in 2000 by adopting The fundamental requirement for establishing the recycling-based society (Radivojević, 2018). Today, the People's Republic of China and the European Union have achieved the most significant progress in the transition from the linear to the circular economy (McDowall et al., 2017; Ghisellini and Ulgiati, 2020). This significant progress is reflected in developed policy regulations and documents, academic publications and media articles, as well as by developing metrics and indicators that monitor the performance of the economy regarding the scale of primary materials use, waste flows, and recycling and circularity. If the CE in the Republic of Serbia is considered, it can quickly be concluded that it is in the initial phase.

In recent years, in Serbia, the benefits of CE for the environment, economic growth, and creation of new jobs have been recognized, which led to some programs that had already begun. Typical examples are preparation of "The strategy for introducing CE into the waste management sector in Serbia", project "Circular Economy Platform for Sustainable Development in Serbia", an "EX-ante analysis of the circular economy effects", and "Road Map for Circular Economy in Serbia". It is necessary to significantly increase awareness and knowledge of the concepts of a circular economy, both for civil servants and for policymakers, so that the government institutions can improve work in this field (Ullstein et al., 2019). In the Republic of Serbia, the most commonly used definition of the CE concept is "the approach in the production that transforms the function of resources – waste from the facilities becomes valuable material in other production processes, and the products themselves can be repaired, reused, or improved instead of being disposed of" (Đureta et al., 2016).

Today, the main focus of academia is to analyze the progress of the transition towards CE (Kalmykova et al., 2018). Suitable methods, tools, and indicators are necessary to quantify the progress of the transition to a circular economy. The problem appears in the fact that, according to the report of Monitoring and advancing the CE transition, there is no recognized way of measuring the effectiveness of the European Union, a country, or even a company's progress of the transition to a circular economy. Also, holistic monitoring tools for supporting such a process do not exist (Saidani, 2018). The methodologies for determining a level of circularity in one production process are not yet sufficiently developed. Namely, only a small number of conducted researches considers circular economy indicators.

The problem that occurs with the lack of tracking and evaluation of circularity was determined by the foundation Ellen MacArthur (EMAF), and having this in mind, the foundation launched and published the project "Circularity indicators" for the first time in 2015. This project aimed to solve the problem of missing indicators in the field of CE and develop a methodology that should estimate the progress of companies in transition from linear into a circular model of running a business. There is a deficit of research in RS on the topic of evaluating the circularity level in the companies so the primary contribution of this research is checking whether methodology launched by EMAF can be adopted in Serbian policy.

3. Problem statement

The adoption of CE on macro and micro levels brings numerous benefits, which can be classified into three primary groups: economic, social, and ecological. However, there are specific barriers for the further development of the CE. Lack of legal regulation in this area is more indicative, as well as the lack of indicators to determine the level of circularity of organizations.

Monitoring or tracking progress towards a CE is a challenging and difficult task because the development of a CE is not limited to certain materials, products, or sectors. It is considered to be a systemic change that affects the entire economy and includes all products and services in different sectors (European Commission, 2018).

So this research aims to provide adoption of the methodology "Circularity indicators" for determining the level of circularity for companies that operate in the Republic of Serbia, as well as defining measures to improve their circular economy model (Pavlović et al., 2018). Based on the mentioned adoption of the methodology, it is achievable to determine the possibility of implementing a CE in companies that are operating in the RS. Namely, according to the level of achieved circularity, it is possible to determine how far the economic business model of the analyzed company is from the circular one and whether the transformation towards the circular model is possible at all.

4. Circularity assessment in companies

The circularity assessment of companies is a highly complex process due to the complexity of determining valid indicators. As a starting point in this research, indicators developed by the European Commission Environment Program partnered with the EMAF and Granta Design on the LIFE+ Project (Cayzer et al, 2017) were used. Publication "Circularity indicators" represents a methodology about defining a material circularity indicator (MCI) of the product/company and about setting the complementary indicators (CIs). The CIs are used to identify relevant risks and impacts of the product, but in this research, CIs were not considered. Based on the publication of "Circularity Indicators", for determining the levels of circularity of the company, at first, it is necessary to assess the circularity level of all products which are produced by the company.

MCI and Linear Flow Index (LFI) indicators are the most significant indicators for determining the circularity level of a company. This is explained by the fact that results of MCI and LFI indicators can be used in the design of new products to take circularity into account as a criterion and input for design decisions. Also, using these indicators companies could be able to parallel different products regarding their circularity.

The MCI is the leading indicator, and it gives information about the product's materials circulate level. The obtained result of the MCI can be in a range from 0 (pure linearity) to 1 (pure circularity). The MCI is evolved to enable companies to understand how far they are from linear to circular model (EMAF, 2015). A higher value of the MCI shows a higher level of circularity.

The material circularity indicator is built from specific data that influence the characteristics of a product (EMAF, 2015; Bracquené et al., 2020):

- mass of virgin raw material used in production process (% of raw, recycled, and reused material),
- mass of unrecoverable waste that is associated with the product's life cycle,
- utility factor that accounts for the length and intensity of the product's use, and
- efficiency of recycling processes.

Based on the previous list, the MCI is estimated from the virgin, reused, and recycled input of the feedstock as well as the reused input. It considers the usage length and intensity, and finally, the end-of-life scenario (Dwek, 2017).

Another important indicator for the circularity level in one company is LFI. LFI measures the proportion of material sourced from virgin materials, ending up as unrecoverable waste, so-called linear part of material flow (EMAF, 2015).

For the development of the circular economy model, it is necessary that the MCI has a value close to 1 and that the LFI has a value approximately equal to 0.

The analyzed circularity indicators (MCI and LFI) have an impact on material sourcing and product design (environmental footprint, energy consumption, level of recycling, water consumption, the price of materials, revenue, etc.).

The methodology for circularity indicators used in this research for the Serbian company presents an adoption of the methodology mentioned above in a specific company. The adoption of a methodology “Circularity Indicators” in companies is significant for its management to identify products that need to improve the circular rate. Table 1 presents the identified strengths and weaknesses of the analyzed methodology.

The following steps show a brief overview of the methodology developed by EMAF (EMAF, 2015).

Step 1. The first step determines the mass of virgin feedstock (V). This mass can be determined by equation 1, where M represents the mass of the final product, while F_R and F_U represent the fraction from recycled and reused sources, respectively:

$$V=M \cdot (1- F_R - F_U) \tag{1}$$

Step 2. The second step consists of determining the mass of unrecoverable waste through a product's material going into a process where the materials are no longer recoverable (W_O). That mass can be specified by equation 2. Similar to the previous case, C_R and C_U represent the fragment of the mass of a product being collected for a recycling process, i.e., for a process of reuse.

$$W_O=M \cdot (1 - C_R - C_U) \tag{2}$$

Step 3. The third step presents the estimation of the efficiency of recycling process (E_C). Equation 3 shows the ratio of the mass of useful recycled components and the mass of all fraction entering the recycling process per period, $\sum m_i(out)$ and $\sum m_i(in)$.

$$E_C = \frac{\sum m_i(out)}{\sum m_i(in)} \cdot 100\,\% \tag{3}$$

Step 4. The fourth step determines the mass of unrecoverable waste generated in the recycling process of a product (W_C). This parameter can be explained by equation 4.

$$W_C = M \cdot (1 - E_C) \cdot C_R \tag{4}$$

Step 5. The fifth step is related to specifying the mass of unrecoverable waste generated when producing recycled feedstock for a product (W_F). The parameter E_F has an essential role in equation 5, and it represents the efficiency of the recycling process used to produce recycled feedstock for the material. E_F can be determined the same way as the parameter E_C.

$$W_F = M \cdot \frac{(1-E_F) \cdot F_R}{E_F} \tag{5}$$

Step 6. The sixth step refers to establishing the mass of unrecoverable waste connected to all life cycles of a product (W), and it is given by equation 6.

$$W = W_O + \frac{W_F + W_C}{2} \tag{6}$$

Step 7. The seventh step determines of linear index flow shown by equation 7.

$$LFI = \frac{V + W}{2 \cdot M + \frac{W_F - W_C}{2}} \tag{7}$$

Step 8. The eight step shows how to determine the utility of a product (X), and it is given by equation 8. X depends on four parameters that are related to the actual average lifetime of a product and an industry-average product of the same type (L, L_{av}) and the actual average number of functional units achieved during the use phase of a product and the industry-average product of the same type (U, U_{av}).

$$X = \left(\frac{L}{L_{av}}\right) \cdot \left(\frac{U}{U_{av}}\right) \tag{8}$$

Table 1
Strengths and weaknesses of methodology that use MCI and LFI indicators (Vercalsteren et al., 2018)

	Strengths	Weaknesses
Methodology “Circularity Indicators”	- The calculation methodology is available. - Repair and remanufacture can be included by adapting the product lifetime and/or component reuse. However, the current methodology does not incorporate a more detailed modelling of repair or remanufacturing. - A simple interpretation of the indicators.	- Only includes material flows, no toxicity, CO_2/energy, scarcity and water. - Circularity indicators on their own are not beatific, it should be seen in the context and be used with complementary indicators. - Software by which these indicators can be automatic used is not open source.

Step 9. Equation 9 represents the way of calculating the MCI_P. It depends on the linear flow index and utility factor *F(X)*.

$$MCI_P^{*}=1 - LFI \cdot F(X) \qquad (9)$$

Utility factor is defined as a function of the utility of the product. In order to improve the utility of the product, EMAF has adopted the function *F(X)* that is similar to equation 10.

$$F(X) = \frac{0.9}{X} \qquad (10)$$

Equation 9 is transformed into equation 10 in order to avoid a possibility that MCI_P value can be negative for products with the mostly linear flow and a utility worse than the average product. Namely, equation 10 is defined in such a way that MCI_P takes, by convention, the value 0.1 for an entirely linear product (i.e., $LFI = 1$) whose utility equals the industry average (i.e., $X = 1$) (Marvuglia et al., 2018; Razza et al., 2020).

Finally, MCI_P is defined as:

$$MCI_P = max\left(0, MCI_P^{*}\right) \qquad (11)$$

Step 10. The material circularity indicator of a company MCI_C can be determined by the MCI_P.

Also, in order to evaluate MCI_C, it is necessary to determine the normalizing factor. It is used to aggregate MCI_P using a weighted average approach. The sum of normalizing factor consists out of the normalizing factor $N_{R(i)}$ of reference products for product range $P_{(i)}$. The index *i* notes to a specific product range or department.

Finally, equation 12 gives the form for calculating the company's material circularity indicator:

$$MCI_C = \frac{1}{\sum N_{R(i)}} \sum (N_{R(i)} \cdot MCI_{P(i)}) \qquad (12)$$

5. Application of "Circularity indicators" methodology in Serbian companies

For the implementation of the CE in companies in the Republic of Serbia, systemic changes in the legislation are needed, as well as the adoption of uniform indicators for monitoring CE at the macro, micro, and nano levels.

5.1. Case study

The company that was chosen for determining the level of circularity in Serbia is "MB INTERNACIONAL". The main activity of "MB INTERNACIONAL" is the production of the packaging made from paper and cardboard and the production of the cupcake liner. In the analyzed company, the procedure of the methodology "Circularity Indicators" was realized in the following order:

Step 1. Primarily, the inventory list was created. The inventory list included a list of all products that were manufactured in the analyzed period. The inventory list of the company "MB INTERNACIONAL" consisted out of 41 products.

Step 2. The second step was to create groups of related products. Products within one group were sorted by the criterion of similarity of material composition (for example, a criterion of similarity can be a type of material and their relative mass).

The analyzed company products range was divided into eight groups:

1) cardboard plates,
2) printed cardboard plates,
3) boxes for rolls and cakes,
4) boxes for cookies,
5) boxes for cakes with an opening,
6) cake pads,
7) cupcake and cookies liner, and
8) party program.

Step 3. The third step involved creating standard accounting information in the form of a table for each group of products. That table consisted of specific data related to every product, such as the name of the product, model of the product, quantity of sold units of the product in the observed period, the unit price of the product, unit mass, the total mass of sold units in the observed period, and the total revenue of manufactured units.

Standard accounting information for each group of products in the analyzed company is shown in Table 2.

Step 4. In the implementation of "Circularity Indicators", the De Minimis rule can be applied. This rule permits the exclusion of particular products from the assessment, in case their share in the total mass of products or the total revenue, given in domestic currency, is lower than 5 % (EMAF, 2015). During the analyzed period of 30 days, the mass of sold products was 14,317.60 kg, while the total revenue, given in domestic currency was 4,896,785.00 RSD. The percentage account indicated that 5 % of the total mass was 715.88 kg, and 5 % of the total sales was 244,839.25 RSD.

Both conditions were fulfilled only for the sixth group of products "box for cookies", so in the following steps that group was excluded from the assessment, because its share in the total mass of products was approximately 4.05 %, while its share in the total revenue equaled 2.69 %.

Step 5. The methodology for determining the circular material indicators at the company level required a reference production approach. Namely, the reference product represented a product, which characterized the whole product range.

Table 2
Standard accounting information for each group of products

Name of product	Model	Units sold	Unit price (RSD)	Unit mass (kg)	Total mass sold (kg)	Total revenue (RSD)
First group of products – cardboard plate					**3,977.86**	**1,077,300.00**
Cardboard plate	1A–1/25	5,650.00	27.00	0.100	565.00	152,550.00
Cardboard plate	2A–1/25	3,800.00	29.00	0.140	532.00	110,200.00
Cardboard plate	3A–1/25	9,760.00	41.00	0.160	1,561.60	400,160.00
Cardboard plate	4A-1/25	2,500.00	57.00	0.210	525.00	142,500.00
Cardboard plate	1B-1/15	3,000.00	27.00	0.080	240.00	81,000.00
Cardboard plate	2B-1/10	3,470.00	30.00	0.090	312.30	104,100.00
Cardboard plate	3B-1/10	2,630.00	33.00	0.092	241.96	86,790.00
Second group of products – Printed cardboard plate					**1,607.09**	**623,750.00**
Printed cardboard plate	2A-1/15	2,945.00	30.00	0.082	241.49	88,350.00
Printed cardboard plate	3A-1/15	9,000.00	43.00	0.120	1,080.00	387,000.00
Printed cardboard plate	3B-1/10	2,800.00	53.00	0.102	285.60	148,400.00
Third group of products – Cupcake and cookies liner					**455.5**	**451,825.00**
RAFAELO liner	1/20/140	150.00	840.00	0.850	127.50	126,000.00
Cookies liner	No1-1/25/100	185.00	800.00	0.850	157.25	148,000.00
Cookies liner	No2-1/25/70	180.00	700.00	0.800	144.00	126,000.00
RAFAELO liner	1/20/4	200.00	45.00	0.015	3.00	9,000.00
Cookies liner	No1-1/25/4	180.00	55.00	0.025	4.50	9,900.00
Cookies liner	No2-1/25/4	125.00	65.00	0.030	3.75	8,125.00
Cupcake liner	M-1/5	310.00	80.00	0.050	15.5	24,800.00
Fourth group of products – Pads					**3,181.1**	**878,770.00**
Pads for rolls	/	8,500.00	29.00	0.095	807.50	246,500.00
Pads for cake	No3	3,120.00	46.00	0.190	592.80	143,520.00
Pads for cake	No5	2,350.00	55.00	0.190	446.50	129,250.00
Pads for cake	No2	2,480.00	50.00	0.160	396.80	124,000.00
Pads for mini roll	MR	4,500.00	27.00	0.075	337.50	121,500.00
Pads for cake	Č-1/1	3,000.00	38.00	0.200	600.00	114,000.00
Fifth group of products – Boxes for rolls and cakes					**3,245.00**	**884,250.00**
Box for mini rolls	/	2,750.00	32.00	0.115	316.25	88,000.00
Cake box	No1	2,000.00	20.00	0.100	200.00	40,000.00
Box for cake	No2	3,000.00	55.00	0.170	510.00	165,000.00
Box for cake	No4	2,500.00	60.00	0.200	500.00	150,000.00
Box for cake	No5	2,500.00	59.00	0.250	625.00	147,500.00
Box for rolls	Š-1/1	6,250.00	47.00	0.175	1,093.75	293,750.00
Sixth group of products – Box for cookies					**580.25**	**131,750.00**
Box for cookies	No2 ½ kg	4,000.00	11.00	0.050	200.00	44,000.00
Box for cookies	No3 1 kg	5,850.00	15.00	0.065	380.25	87,750.00
Seventh group of products – Boxes for cookies/cakes with an opening					**547.5**	**254,000.00**
Box for cookies	No1 ½ kg	2,500.00	16.00	0.035	87.50	40,000.00
Box for cookies	No2 1 kg	4,000.00	20.00	0.045	180.00	80,000.00
Box for cake	No3	2,000.00	67.00	0.140	280.00	134,000.00
Eight group of products – "Party" program					**723.3**	**595,140.00**
Birthday hat 1/6	1/6	3,900.00	44.80	0.075	292.50	174,720.00
Birthday cup 1/10	1/10	3,120.00	45.00	0.055	171.60	140,400.00
Party set 3/10	3/10	1,890.00	58.00	0.080	151.20	109,620.00
Birthday trumpet 1/6	1/6	2,400.00	71.00	0.045	108.00	170,400.00
Total					14,317.60	4,896,785.00

In this assessment, the following products were selected as the reference products:

- Cardboard plate 3A-1/25,
- Printed cardboard plate 3A-1/15,
- Cookies liner No 1/25/100,
- Cake pads /,
- Box for roll Š-1/1,
- Box for cake No3,
- Birthday hat 1/6.

Products whose demand was the highest in the observed period were chosen for the reference products.

Step 6. In this research, for each reference product, the table "bill of materials" was created.

A bill of materials is a list of the parts or components that are required to build a product (EMAF, 2015). That list included data on materials and mass of material which went into the process of production. A bill of materials provided information related to recycled or reused materials and also information about the predicted share of materials that could be recycled or reused after the phase of product use. The bills of materials for the seven reference products are shown in Table 3.

Table 3
Bill of materials for the reference products

Component	Material	Mass [kg]	% recycled feedstock	% reused feedstock	% recycled after use	% reused after use
			Cardboard plate 3A-1/25			
Plate	Cardboard	0.1575	97.00	0.00	100.00	0.00
Label	White paper	0.0005	0.00	0.00	100.00	0.00
Bag	PE foil	0.0020	25.00	0.00	100.00	0.00
			Printed cardboard plate 3A-1/15			
Plate	Card board	0.1145	97.00	0.00	100.00	0.00
Label	White paper	0.0005	0.00	0.00	100.00	0.00
Bag	PE foil	0.0020	25.00	0.00	100.00	0.00
Deco layer	Color	0.0030	0.00	0.00	0.00	0.00
			Cookies liner No 1/25/100			
Cookies liner	Pergament paper	0.750	0.00	0.00	0.00	0.00
Label	White paper	0.0005	0.00	0.00	100.00	0.00
Deco layer	Color	0.0095	0.00	0.00	0.00	0.00
Box	PET	0.090	0.00	0.00	100.00	100.00
			Cake pads /			
Pad	Cardboard	0.0919	85.00	0.00	100.00	0.00
Label	White paper	0.0005	0.00	0.00	100.00	0.00
Deco layer	Deco Pe foil	0.0008	0.00	0.00	50.00	0.00
Contact layer	Contact glue	0.0008	0.00	0.00	0.00	0.00
Bag	PE	0.0010	50.00	0.00	100.00	100.00
			Box for roll Š-1/1			
Box	Cardboard	0.1715	97.00	0.00	100.00	0.00
Label	White paper	0.0005	0.00	0.00	100.00	0.00
Contact layer	Contact glue	0.0020	0.00	0.00	0.00	0.00
Bag	PE	0.0010	50.00	0.00	100.00	100.00
			Box for cake No3			
Box	Cardboard	0.1365	97.00	0.00	100.00	0.00
Label	White paper	0.0005	0.00	0.00	100.00	0.00
Contact layer	Contact glue	0.0020	0.00	0.00	0.00	0.00
Opening	PE foil	0.001	25.00	0.00	100.00	0.00
			Birthday hat 1/6			
Hat	Cardboard	0.0695	97.00	0.00	100.00	0.00
Label	White paper	0.0005	0.00	0.00	100.00	0.00
Deco layer	Color	0.0020	0.00	0.00	0.00	0.00
Bag	PE	0.0010	50.00	0.00	100.00	100.00
Contact layer	Contact glue	0.0020	0.00	0.00	0.00	0.00

As already emphasized, the circularity at the level of the reference product was determined first. Therefore, the share of materials used in the production process for a reference product should be calculated as well as the possible level of recycling and reuse of the material after the product use phase. Namely, these percentages were expressed in table 3, but especially for each component, which was integrated into the product mass. The necessary data for the product was determined using the arithmetic mean given in equation 13.

$$\overline{X} = \frac{\sum_{i=0}^{n} x_i}{n} \tag{13}$$

In the previous equation, the numerator represented the sum of all the results in the sample, while the denominator represented the number of samples.

So, according to this statistical technique, Table 3 was then transformed into Table 4.

Step 7. In this step, the real lifetime of the reference product should be defined as well as the average lifetime of a similar competitor's product, the actual number of functional units, and the average number of functional units of a similar competitor's product.

If it was not possible to ensure a reliable estimation of a lifetime and the number of functional units, it was necessary to acknowledge that these parameters were equal and that their ratio equaled 1 (EMAF, 2015). In this case, all the mentioned parameters were equivalent to 1.

It is important to emphasize that parameters of recycling process efficiency at the end of life and efficiency of the recycling for feedstock were established according to the report published by the "Deutsche Gesellschaft für Internationale Zusammenarbeit" (GIZ) (Bobić, 2019).

Step 8. Using the mathematical formulas from chapter 4, MCI_P and LFI_P were determined for each reference product. The assessment was done using Microsoft Excel software. The example of a table which was used for evaluation is shown in Table 5.

Table 4
Bill of materials for the reference products

Reference product	Mass	% recycled feedstock	% reused feedstock	% recycled after use	% reused after use
Cardboard plates 3A-1/25	0.16	40.67	0.00	100.00	0.00
Printed cardboard plate 3A-1/15	0.12	30.50	0.00	75.00	0.00
Cookies liner No 1/25/100	0.085	0.00	0.00	50.00	25.00
Cake pads /	0.095	27.00	0.00	70.00	20.00
Box for roll Š-1/1	0.175	36.75	0.00	75.00	25.00
Box for cakes with an opening No 3	0.140	30.50	0.00	75.00	0.00
Birthday hat 1/6	0.075	29.40	0.00	60.00	20.00

Table 5
Evaluating MCI of Reference product: Cardboard plates 3A-1/25

Metrics for core sustainability indicators	Data	Units
Product total mass (M)	0.16	kg
Product mass from recycled feedstock (Fr)	40.67	%
Product mass from reused components (Fu)	0.00	%
Product sent for recycling at end of life (Cr)	100.00	%
Product sent for reuse at end of life (Cu)	0.00	%
Efficiency of recycling process at the end of life (Ec)	40.00	%
Efficiency of recycling process for feedstock (Ef)	55.00	%
Product lifetime **OR** Product use (L or U)	1.00	Yr
Industry average lifetime **OR** industry average use (Lav or Uav)	1.00	Yr

Linear Flow Index (LFI)	**0.57**
Material Circularity Indicator (MCI)	**0.49**

Step 9. The ninth step involved defining normalization factors which were determined for every reference product. In this case, the normalizing factor was used to aggregate material circularity indicators for the reference product. For that purpose, the mass of the product group was taken as a normalization factor. After completion of the steps eight and nine, it was possible to define precisely the circularity levels for the reference products. Results of MCI_P are shown in Table 6.

Step 10. The mathematical combining of MCI_P was done at the level of the product in order to calculate the level of circularity of the company MCI_C. Above mentioned combination was done according to equation 12 and the final obtained result was 0.47.

Results of calculations are shown in Figure 1, indicating that the company operated using a business model which was in the between linear and circular model.

Table 6
Results of MCI_P for reference products

Name of product's group	Mass of the group of product [kg]	MCI_P
Cardboard plate	3,977.86	0.49
Printed cardboard plate	1,607.09	0.39
Cupcake and cookies liner	455.50	0.32
Pads	3,181.10	0.46
Boxes for rolls and cakes	3,245.00	0.53
Boxes for cakes with an opening	547.50	0.39
Party program	723.30	0.44

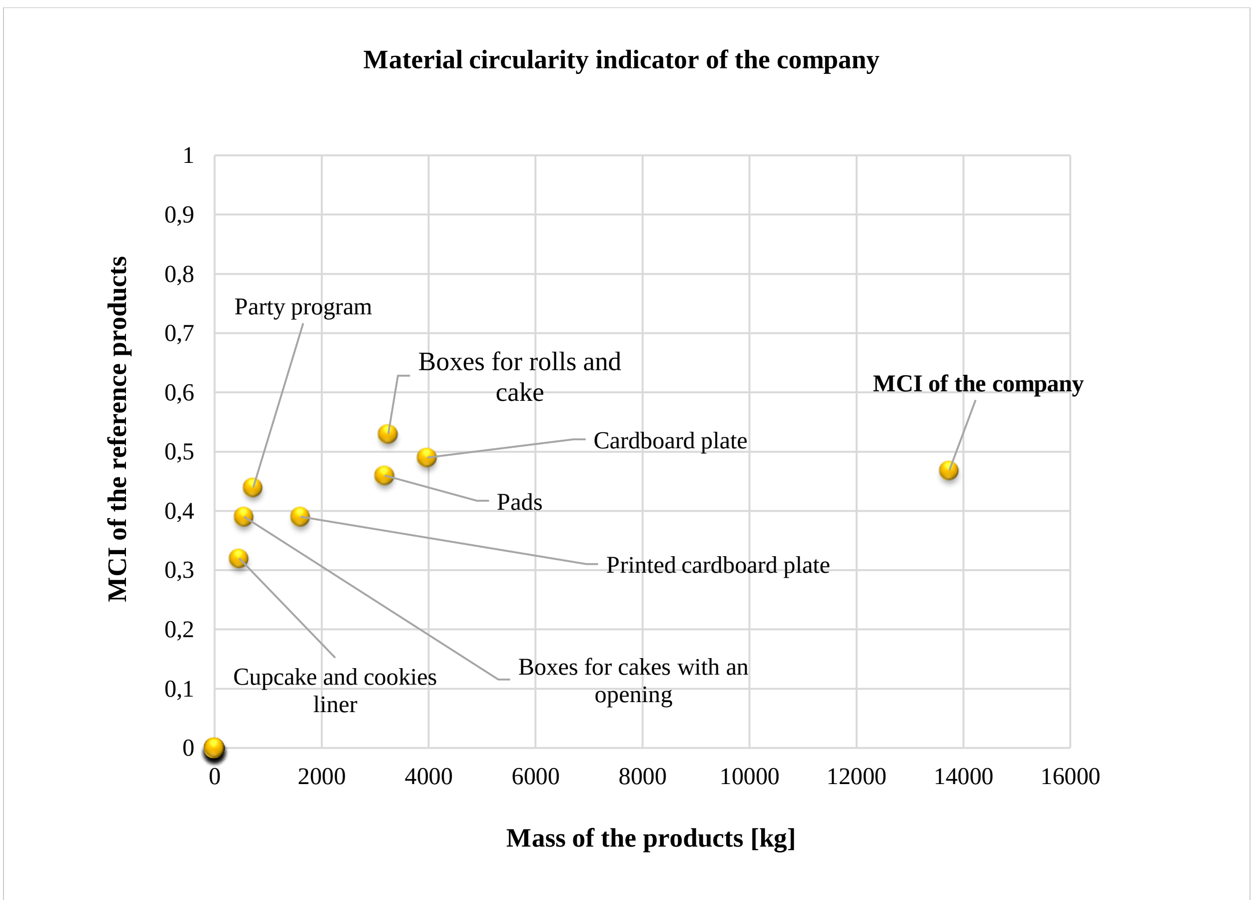

Figure 1. Results of circularity level in the analyzed company (Pavlović et al., 2018)

6. Measures for improving circularity indicators in analyzed company

To achieve complete implementation of the circular economy, changes in the way of production and consumption of the products are necessary.
Promoting a CE in companies that operate in Serbia is not only a key for Serbia's integration into the EU, but also, it is an opportunity for innovation, efficient use of resources, participation in numerous researches, and financial programs, etc. The CE seeks a different manner related to waste management and recycling, as well as increased discipline in respecting the guidelines for such directives and improved monitoring of waste flows.

The obtained value of the Circular Indicator for “MB INTERNACIONAL” was approximately 0.47, which means that the company can proceed with specific measures, which will improve the circularity level.

Figure 2 shows the necessary steps that should be taken in order to transform the company’s business model from a linear to a circular.

So far, the company “MB INTERNACIONAL” has not performed any actions related to the development of a circular economy. Still, the company’s main activity is such that it uses organic raw materials in its production process, which affects a higher level of circularity. After the performed evaluation of circularity, it is necessary to carry out “benchmarking”, i.e., to explore and discover the advantages and examples of good CE practices of competing companies, which should be applied afterward.

Switching from a linear business model to a circular is a complex process, which involves constant monitoring and improvements. Company “MB INTERNACIONAL” can implement specific measures to improve the level of circularity, which can be low-budget or high-budget.

The first reason for the development of the CE in the company is the phenomenon of improvement of the brands’ reputation, and thereby, the company becomes more competitive in the market.

Low-budget measures may even include the introduction of eco-design into the company’s business model. Eco-design implies the creation of such a product, which, during its life cycle, has a minimal negative impact on the environment. This type of product design is reflected in the design of new, more energy-efficient products, which will be produced from 100 % biodegradable materials, and materials that allow the reuse of these products, or recyclable materials.

Due to environmental protection, the company “MB INTERNACIONAL” has to promote the recycling of paper and paperboard, because it saves forests, energy used for the production process, and the most important, there is no generation of methane.

However, for the analyzed case study, there was a recycling problem after the product use phase. The company carries out the production of cardboard packaging and the packaging used for storing food, so after the use phase the products are damaged by the rest of the food, which makes the recycling process more difficult. Solving this problem can be composting waste paper or paperboard.

Another way of improving the CE in the company is the introduction of an efficient environmental management system.

Also, the company must optimize the generated amount of waste in the production process.

One of the high-profile measures that can improve the company's circularity is a purchase of a hydraulic press for baling fibrous materials. That way, the waste would be treated within the company itself, and then the company could sell the newly created product to factories, which mainly manufacture materials from recycled paper or cardboard waste.

Implementing the CE in the company “MB INTERNACIONAL” is possible, as well as improvement of the same. For the implementation of the CE, it is necessary to identify critical phases of the product’s life cycle in the analyzed company. That can be done by using the “Circular Economy Toolkit – CET.”

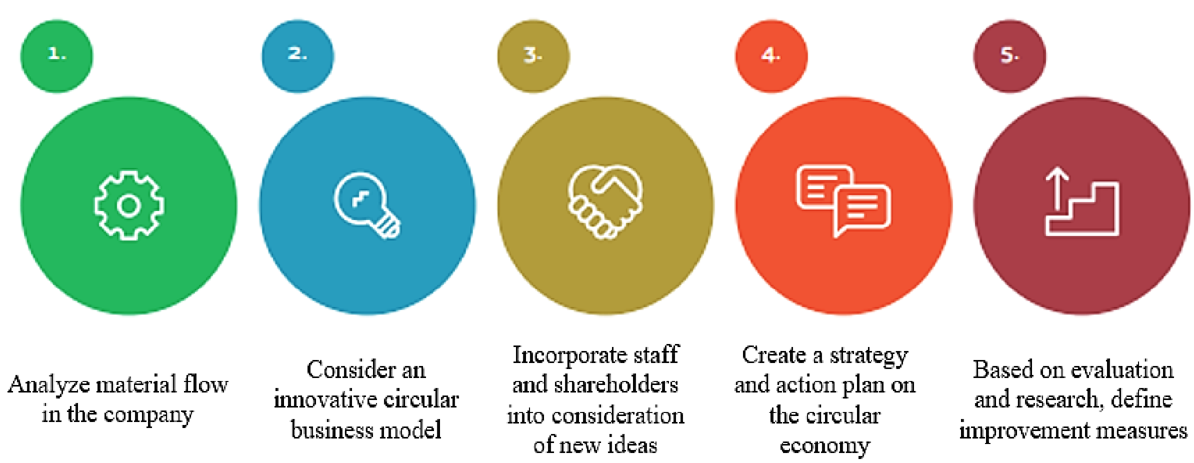

Figure 2. Path of transition to a CE in one single company

CET is developed at the University of Cambridge. The CET is an assessment tool to identify the potential improvement of the products' circularity (Evans and Bocken, 2014). This is a fast online tool which the companies use to evaluate the improvement potential of products towards circularity.

For the analyzed company, the CET was done, and the outcome is shown in Table 7.

CET can identify the most important and critical phases of the product's life cycle. As stated, companies have insight into the areas with a high, medium, or low potential for the improvement of the circularity activities, as shown in Table 6 for the "MB INTERNACIONAL" company.

Transformation to CE requires changes in number of organizational dimensions, such as value creation, customer segments, and innovation activity (Primc et al., 2020). In order to develop and sharpen the concept of a circular economy, it is necessary to affect the consumers' way of thinking and then change the business model. Consumers have an essential role in the CE, which leads to a conclusion that the transition of a consumption model of a product is of great importance and it must be carried out first.

Table 7
Result of the Circular Economy Toolkit

Improvement Area	Improvement Potential
Reduce materials	High opportunity
Optimize materials	Medium opportunity
Industrial Symbiosis	High opportunity
Usage	High opportunity
Maintain/Repair	High opportunity
Reuse/Redistribute	High opportunity
Refurbish/Remanufacture	Medium opportunity
Product Recycling	Medium opportunity
Product as a Service	Medium opportunity

7. Conclusion and future work

The circular economy model is an essential model for managing resources more efficiently. The researched model is useful for creating a regenerative economy that has positive economic and environmental effects. The application of a circular economy model implies a transformation of the whole system of waste management. It is a systematic change that is necessary for the development of the Republic of Serbia in the environmental field. The main advantage of the implementation of the CE in the Republic of Serbia can be explained by the fact that it creates resource efficiency, promotes renewable energy, and enables cleaner production, which moves towards zero waste.

Contrary to that, the main disadvantage of the proposed methodology can be noted as the lack of legislation in the field of the CE. Simultaneously, that is the main reason why companies in Serbia still do not admit the CE as a chance for future development.

After implementing the methodology "Circularity Indicators" in the specific company that operates in Serbia, it is concluded that the CE was not total newness - because the obtained value of *MCI* was 0.47. This means that the company operates according to the economic model, which is between linear and circular.

Using specific measures that can be low-budget or high-budget, the analyzed company can improve the current economic model.

It has been said before that there is a deficit of research on the topic of evaluating the circularity level in the companies in Serbia, so this research checked if it was possible to adopt methodology launched by EMAF in Serbian policy. Obtained results in the specific company led to the general conclusion that methodology could be implemented in companies that operate in Serbia because their management had all the data needed to determine the level of circularity.

The methodological approach for determining the circularity at the company's level is essential for numerous estimates and indicators. The most common performance assessment of an organization is done using the so-called key performance indicators (KPIs). The KPIs should be related to the company's strategic goals (finance, market, processes, and people) but also considering the circularity goals.

Further research in the CE field should be carried out in a more significant number of companies. Also, it is proposed to develop or implement new indicators, which will determine the circularity level. For developing new projects and researches in fields of the CE in the Republic of Serbia, it is needed to train industrial experts in product circularity assessment, as well as establish indicators and tools for measurement and monitoring of circularity.

Acknowledgement

This research has been supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (Project III 42013). Work of Natalia Sliusar and Nemanja Stanisavljevic is financially supported by the Government of the Perm region, Russia, within the framework of research project no. C-26/174.6.

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Određivanje indikatora cirkularne ekonomije – studija slučaja kompanije „MB INTERNACIONAL''

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INFORMACIJE O RADU

Primljen 30 jun 2020
Prihvaćen 25 septembar 2020

Originalan rad

Ključne reči:
Cirkularna ekonomija
Indikatori cirkularne ekonomije
Indikator cirkularnosti materijala
Održivi razvoj

Proširena i recenzirana verzija rada - rad prezentovan na simpozijumu 3. ICQL

I Z V O D

Cirkularna ekonomija (skraćeno CE) je trenutno svetski popularan koncept koji bi trebalo da osigura održivi razvoj i efikasnost resursa. Koncept je zasnovan na teoriji potrošnje i upotrebe resursa u postupku proizvodnje na način koji utiče na ograničavanje štetnih efekata na životnu sredinu. Ovaj koncept istovremeno stvara dodatnu vrednost proizvoda i omogućava njegovo ponovno korišćenje. U Republici Srbiji je ideja o cirkularnoj ekonomiji još uvek nova i nerazvijena. Stoga, cilj ovog rada je da istraži mogućnost primene CE u kompanijama koje posluju u Srbiji usvajanjem metodologije koja je već razvijena u Evropskoj uniji. Ovo istraživanje je sprovedeno u kompaniji „MB INTERNACIONAL" koja proizvodi kartonsku ambalažu gde je praćen proizvodni postupak. Dobijena približna vrednost indikatora cirkularne ekonomije je iznosila 0,47, što ukazuje na to da kompanija ima odličnu šansu za potpunu primenu modela cirkularne ekonomije u poslovanju uz primenu posebnih mera. Niskobudžetne i visokobudžetne mere koje bi doprinele povećanju nivoa cirkularnosti u analiziranoj kompaniji su takođe predstavljene u ovom radu.

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Recycling and Sustainable Development

RSD

Online ISSN 2560-3132
Print ISSN 1820-7480

Biowastes as a source of extracting chitin and chitosan for biomedical applications

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ARTICLE INFO

Received 17 June 2020
Accepted 30 September 2020

Review article

Keywords:
Biowaste
Chitin
Chitosan
Wound Healing
Drug Delivery
Tissue Engineering

ABSTRACT

Biomaterials are designed to interact with biological systems in aid to wound healing, regeneration of tissue, mechanical support, and drug delivery to eventually improve current therapeutic outcomes. The adoption of biomaterials is increasing constantly in health care practices by making it more biocompatible and non-toxic under physiological conditions. These adoptions have been associated with improvements in therapeutic outcomes across the population, however, the dosage of therapeutics needed to successfully treat a disease is generally different for each individual and relies a lot on experiences of consultant doctors. Many times, it leads to human errors in deciding on drug doses, un-fit implants and explants and eventually adverse effects or less positive effects. The personalized medicine and devices bring forth the idea that the medicine should be tailored for a patient based on various characteristics, such as gender, age, genetic makeup, and lifestyle. These personalized medicine approaches include type of drugs, activation methods, nano-assemblies, biomedical devices, etc. Among these approaches, personalized biomedical devices have become popular with the advent of 3D printing technologies, which can make customized implants for each patient with minimum price, limited time, and high accuracy. Personalized biomedicine also involves designing of drug to cater the need of an individual with minimum side effects. In this review an effort has been made to introduce different aspects of customized biomedical agents like therapeutic biomolecules, nanomedicine, implants, and explants. This comprehensive review of literature indicates that use of 3D printing technology in producing drug releasing, biodegradable personalized implants could be better therapeutic solution for a range of medical conditions.

1. Introduction

Biomaterials can be synthetic or obtained from natural sources for use in various applications including cure of various diseases like Alzheimer's disease (Hamedi et al., 2018), dental implants, prosthetic limbs (Williams et al., 1990), or for making scaffolds like collagen modified with lysine or hydroxy lysis (Sosnik and Sefton, 2005) or PEGylated fibrinogen (Ben-David et al., 2013). Thus, these biomaterials are very expensive because of the non-availability of the resources required for making the product or complex science and technology required to

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doi: 10.5937/ror2001023Y

develop (Bhat and Kumar, 2013). Their use in important biomedical interventions makes them vital for production on larger scale. Synthetic polymers were taken as source for biomaterial industry, but disclosures of their negative implications hard-pressed the researchers for course corrections. The problems of using synthetic polymers are non-biodegradability, associated toxicity, and non-biocompatibility. These concerns were behind the use of natural biopolymers as a very logical solution. Chitin and chitosan are two of the naturally available biopolymers which are known to overcome many of the reported shortcomings of synthetic polymers and can be extracted with higher benefits (Bakker et al., 1999). Additionally, chitin and chitosan are commercially viable because of their biocompatibility, biodegradability, chelation, non-toxicity and incredible adsorption power. All these attributes have allowed chitin and chitosan to find various uses in different kinds of industry like biotechnology, medical, food, and pharmaceutical to name a few. But they have established a place for themselves in biomedical chain of things. They have found their applications in wound healing, drug delivery, bio adhesives, etc. (Dhillon et al., 2013; Zhou et al., 2014; Wijesena et al., 2015)

Interestingly, after cellulose, chitin is the most inherent polymer in nature. Chitin and its derivatives have extravagant commercial value because of their diversified uses in biology and medical industry. However, some of its properties, like crystallinity and insolubility in water, hinder some of its applications. But, the use of its derivatives like chitosan, chito-oligosaccharides, etc. can be used to overcome those drawback (Kumar, 2000; Younes and Rinaudo, 2015; Ghormade et al., 2017; Yadav et al., 2019). The mentioned benefits of chitin and chitosan make them desirable polymeric materials for big scale industry productions and require better learning about their economical sources. This review article provides information regarding chitin and chitosan, that is, their extraction from the waste produced by different industries and advanced applications.

The chitinous waste are generally discarded by either flaring them or disposing it at a landfill. These methods are very hazardous to the environment (Xu et al., 2013). These wastes can be used to produce chitin and chitosan which are used further for different purposes like biomedical materials, fermentation, sewage treatment, etc. Chitosan is made up of both deacetylated and acetylated subunits of *D-glucosamine*. These two entities are linked by *β-(1,4) glycosidic* linkage. The linear polysaccharide is produced by de-acetylation where an acetate group and $–NH_2$ group is obtained by acetamide group's hydrolysis. *N-acetylglucosamine* ratio determines the degree of acetylation in chitosan (Fig. 1). This ratio is higher in chitin compared to chitosan (Ramírez et al., 2010; Viarsagh et al., 2010). Thus, chitin and chitosan although being extremely similar structurally, can be used for different purposes based on required properties of final products. A large availability of these biowastes make them ideal candidate for extraction and use as industrial raw material benefitting the environment and providing economical starting material for various biomaterial industries.

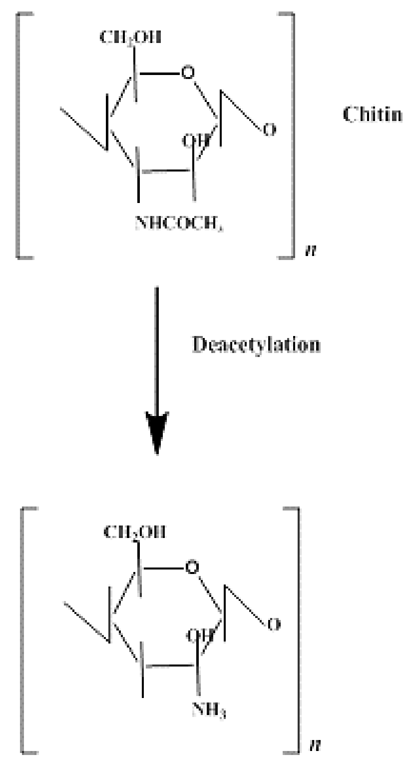

Figure 1. Conversion of chitin to chitosan by deacetylation. *N-acetamido* bonds in chitin monomer (*2-(acetylamino)-2-deoxy-d-glucose*) hydrolysed by chitin deacetylase to produce chitosan

2. Characteristics of Chitin

Chitin is present in the environment in three different forms which are crystalline in nature, namely *α*, *β*, and *γ*. These three different forms vary in the degree of de-acetylation (Aam et al., 2010). The *α-chitin* is generally present in yeast and fungal cell wall, insect cuticles, shrimp cells, etc. Polysaccharides are arranged in such a way that they are present in antiparallel fashion. This allows them to form maximum number of bonds. *α-chitin* is the main form of chitin present in the environment. Crystallinity index of these fibrils is approximately 80 % (João et al., 2015). *β -Chitin* exists in squid pens, some protozoa, and seaweeds. A diatom called *Thalassiosira fluviatilis* excretes a pure form of *β-chitin*. Polysaccharides are organized in a parallel manner. The crystalline index of this form of chitin is 70 %. Due to this arrangement, the distance between the chains increases, which make this structure more reactive and easily soluble in solvents (João et al., 2015). *γ-Chitin* is made up of both *α* and *β* forms in a way that two parallel polymer units are organized alternatively with one anti parallel unit.

3. Generation of Chitinous wastes from bio-industries

As the population of the world is increasing, waste production has inevitably increased. The by-products of one industry can be used as a substrate to produce some other product. However, these by-products are treated as wastes and either dumped in sea or disposed at a landfill where they are not easily degraded. These wastes can be used for further isolation of chitin which can be further used for different purposes (Xu et al., 2013).

3.1. Seafood industry

Dumping of waste in oceans is one of the major factors leading to the environmental pollution where sea food industry is the major source of chitinous wastes. Chitin is generally present in all the aquatic crustaceans, shrimps and constitutes the major amount of dry weight in shrimps. Approximately 10^{12} - 10^{14} tons of chitin are manufactured annually by using the marine life forms. This amount of chitin should be enough to use as a raw material to produce different biomaterials or for other useful purposes. Chitin is generally extracted using chemical methods from such wastes, but biological methods are also used to some extent (Cauchie, 2002).

3.2. Silkworm industries

Silkworms are an unorthodox source of chitin and chitosan obtained from industries. The chrysalides of the silkworm (*Bombyx mori*) are the adult form of the larvae which form the silk threads and form cocoon. These chrysalides are a by-product of the silk industry. They are very cheap and easily available. Chitin constitutes around 20 % of the structure of silkworm. The yield with which chitosan is extracted from the chrysalides is very low but the purity of the chitin isolated is very high. (Paulino et al., 2006)

3.3. Honeybee industry

Obtaining chitin from honeybees is a very difficult process but large amount is generated for industrial purposes. Chitin is bound to different melanin and sclerotin-like proteins. They are a rich source of chitin and chitosan. In 2002, Russian Federation had 3.44 million bee colonies which constitute upto 3.5 - 4 kg of honeybees. This produces a lot of chitinous waste annually (Nemtsev et al., 2004).

3.4. From insect pests

Insects have grabbed a lot of attention as good source of chitin, and consequently chitosan. Cuticle present on the insect surface has less inorganic material than the crustaceans along with chitin. This makes the isolation of chitin much easier and convenient. For e.g. *Holotrichia parallela* is a beetle species which is a pest in China. Annually, this pest is caught in China to reduce the problem of pests. Chitin was isolated from this beetle quite effectively (Liu et al., 2012).

4. Methods of Chitinaceous material isolation from waste

The process of extraction of chitin and chitosan from bio-wastes can be done by chemical and biological procedures. During isolation of chitin from the natural sources, many different variables like molecular weight, degree of acetylation, purity, etc. are taken in consideration. Chemical methods are not eco-friendly and cause damage to the environment. Chemical interactions can also change the physical and chemical properties of chitin. Biological methods help in alleviating these problems. They are still in development phase and, therefore, not as popular as chemical methods (Abdou et al., 2008; Schmitz et al., 2019). Involvement of these processes in various industry based biowaste can be evaluated to understand the loss-benefit scenarios.

4.1. Seafood industry

Seafood industry is the primary source from which commercial chitin is being extracted. The chitin is drawn out from shells, crustaceans, and other sea life. Chitin is the major part of the waste produced by this industry. Chitin is extracted chemically as well as using biological methods (Fig. 2b).

A. Chemical method

Shells which were obtained from different sources can dry after washing. They are then crushed into fine particles (Islam et al., 2004). Chemical withdrawal of chitin is done in three steps including (a) Deproteination (b) Demineralization, and (c) Decoloration.

(a) Deproteination

This step includes the removal of proteins from the chitin sample. These proteins are attached covalently to the biopolymer. This step helps in disrupting the chemical bonds that are present between these two components using chemical reagents like NaOH. This step is important from biomedical point as the protein content is a major reason behind the allergic reaction that are caused in the organisms. Different types of chemicals in different concentrations were used for this step to increase the efficiency. It also leads to hydrolysis of biopolymer and deaceylation of chitin which decreases the molecular weight. It can also change the properties of the chitin or chitosan. (Yadav et al., 2019)

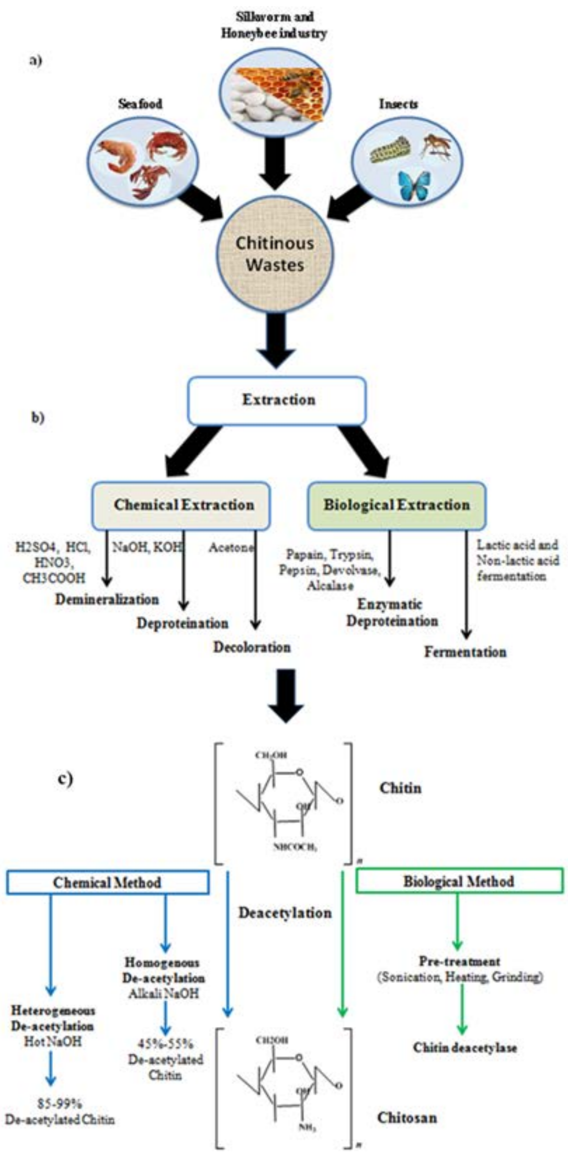

Figure 2. a) Extraction of chitin from chitonous waste; **b)** Extraction methods of chitin from biowastes using chemical methods like NaOH, H_2SO_4 etc. and biological method, eg: trypsin, alcalase etc; **c)** reaction mechanism of chitosan production from chitin using chemical and biological method

(b) Demineralization

Demineralizaton refers to the removal of minerals like $CaCO_3$. It is usually done by treating the polymer with acids like HNO_3, HCl, CH_3COOH, etc. However, HCl is preferred for this process. In demineralization $CaCO_3$ is removed from the sample by converting it to CaO and CO_2. (Percot et al., 2003)

$$2HCl + CaCO_3 \rightarrow CaCl_2 + H_2O + CO_2 \qquad (1)$$

(c) Decoloration

This step is usually done only when colorless product is required. Generally, an organic solvent like acetone is used to separate pigments from the samples. (Mohammed et al., 2013)

B. Biological method

Biological method used for the chitin isolation revolves around the idea of 'Green chemistry'. The use of microbes and enzymes for chitin recovery is involved in biological method of chitin recovery. Generally, a chemical method is used for chitin isolation but the process is energy consuming (Dhillon et al., 2013) and cause negative effects on physico-chemical properties of the biopolymer. In comparison, biological methods are cleaner, more economical, and they allow the isolation of chitin with desired properties. (Khanafari et al., 2008) The two methods generally used to produce chitin and chitosan biologically are:

(a) Enzymatic deproteination

Proteases are enzymes that cleave the peptide bond present between two amino acids and thus can be used for the deduction of proteins during chitin extraction from the waste produced by seafood industry. Proteases like papain, alcalase, pancreatin, etc. can be implemented for the isolation of chitin. The use of proteases allows minimal de-acetylation and depolymerisation during the segregation of chitin. Protein accessibility to the proteases can be increased by doing demineralization step before the deproteination. Unrefined and purified enzymes both can be used for the purpose of deproteination. The use of crude enzymes from fish can bring the cost of extracting chitin down. The study with proteases on demineralised shrimp waste was done and it was found that the chitin extracted was satisfactorily pure (Rao and Sharma, 1997). Biological processes are environmentally and economically advantageous but efficiency of chemical deproteination is still higher. Around 5 - 10 % of protein remains bound to chitin even after treating with different enzymes. Therefore, additional NaOH treatment is needed for complete removal of protein attached to chitin.

(b) Fermentation

The cost of enzymes can be significantly high, especially when purified enzymes are being used. This demands an alternative method by using microbes which can be more economical and efficient due to modification possibilities in microbial population. The selection of microbes is based on the fermentation which can be single or two-stage, successive fermentation or co-fermentation. (Arbia et al., 2013) Microbial fermentation for production of deproteination enzymes is divided into two broad types: lactic acid and non-lactic acid fermentation.

Lactic acid fermentation - Crustacean shells in presence of *Lactobacillus sp.* are generally used for this kind of fermentation. Lactic acid produced during this process by feeding on glucose further decreases the pH and does not allow any spoilage bacteria to grow. *Lactobacillus sp.* produces lactic acid along with a variety of proteases. The lactic acid fermentation's productivity relies on various components like microbial composition and quantity of inoculum, pH of the system during the process, temperature, time, carbon source, and its concentration, etc. (Prameela et al., 2010)

Non-lactic acid fermentation - Bacterial types generally used for this kind of fermentation are *Bacillus sp*., *Aspergillus sp*., etc. (Mahmoud et al., 2007; Sini et al., 2007) Different physico-chemical conditions are known to be affecting this fermentation and thereby deproteinization and demineralization proficiency. Ghorbel-Bellaaj et al. (2012) isolated proteases from *P. aeruginosa* and found that enzyme-substrate ratio and varying reaction time could influence the deproteinization efficacy of protease.

4.2. Silkworm industry

The chrysalides of the silkworm can be used for extraction chitinous materials two distinct methods post lyophilization. One of the methods is by using a closed reactor made of Teflon. The alternative method utilizes an open system of heating plate. HCl is added to dried chrysalides for eradication of catechols like Mg, Ca, etc. Filtration is done to isolate the residue which is then washed with deionized water to level the pH of the solution. These residues are then treated with NaOH to erase any trace of proteins. The treatment of the chrysalides is performed at higher temperature because of the presence of fat contents. If this reaction is performed at room temperature, it leads to saponification which makes the filtration process practically impossible. Reaction yield has been found out to be higher in an open reactor along with impurities, whereas chitin obtained from the closed reactor is reported to be almost free from any form of impurity, but with reduced overall yield (Paulino et al., 2006).

4.3. Honeybee industry

Dry dead bees were isolated and suspended in a specific amount of water w.r.t the bees. NaOH was put into the solution at high temperature to hydrolyze the protein present in the sample mixture. The solid component obtained is filtered followed by discoloration using H_2O_2. (Nemtsev et al., 2004)

4.4. Insect and pests

Various insects and pests can be used as source for isolation of chintinous materials. Isolation process involves starvation of insects/pests for 48 hours to remove any kind of food material present in the gut. Further they are cleaned with water and killed by freezing. These frozen corpses are then thawed and dried at 50 °C for next two days. These dried corpses are then crushed to a powder to store at 4 °C in airtight containers. This powder is then treated with HCl for demineralization, followed by rinsing with water to attain a neutral pH. The demineralized chitin is then treated with NaOH to eliminate the protein which is bound to chitin. Decolorisation is done by the use of potassium permanganate. (Chang et al., 2001; Liu et al., 2012)

5. Chitosan Production from Chitin

Chitin can be transformed into chitosan using either chemical or biological methods (Fig. 2c). Due to the requirement of mass production and procedures, chemical method is the one which is preferable, but high quality and environmental suitability makes biological method a better choice. (Tokuyasu et al., 2000; Philibert et al., 2017)

5.1. Chemical method

In chemical method of chitosan production from chitin, either acid or alkalis can be used for the process of de-acetylation but acid sensitivity of glycosidic bonds make alkalis more preferable (Hajji et al., 2014). De-acetylation of chitin can be generally classified into heterogeneous and homogenous de-acetylation processes. In heterogeneous de-acetylation, hot NaOH is added to the chitin for few hours and an insoluble substrate, chitosan, is obtained. It is generally obtained in the form of around 85 - 99 % of de-acetylated chitin. In the homogenous de-acetylation, chitin is treated with alkali NaOH for three hours at 25 °C. After this, chitin is put in 0 °C which is achieved with the help of crushed ice. Chitosan obtained with the help of this process is 45 % - 55 % de-acetylated chitin. If this process is continued for around 580 hours, then it leads to formation with 90 % degree of de-acetylation. The acyl group present in chitosan are generally homogenously dispersed. (Aiba, 1991)

Under heterogeneous conditions, de-acetylation reactions lead to bumpy division of *D-glucosamine* and *N-acetyl glucosamine* in the polymer. Because of this, degree of aggregation, solubility differs in aqueous solution leading to change in the characteristics. Additionally, degree of acetylation, molecular weight, etc. can change due to changes during the chitosan preparation (Berger et al., 2005). Temperature and processing time are two of the most important factors that affect the degree of acetylation and molecular weight of produced chitosan (Rege and Block, 1999). Molecular weight and de-acetylation of chitosan are affected by concentration of NaOH too. (Tsaih and Chen, 2003)

5.2. Enzymatic method of converting chitin to chitosan

The enzymatic method for genesis of chitosan from chitin is done using enzyme chitin deacetylase. It hydrolyses the acetoamido present in *N-acetyl glucosamine* units of chitin and produces acetic acid and glucosamine units. It is a member of carbohydrate esterase. Different types of chitin deacetylase were isolated from different bacteria (*V. cholera*), fungi (*A. niger*, *M. racemosus*), insects (*D. melanogaster*, *Apismellifera*), and enzymatic deacetylation and then studied. Anyhow, chitin deacetylase is perceived to be not as successful on innate chitin which is insoluble and crystalline in environment. To increase the access of acetyl group to chitin deacetylase, pre-treatment is done which includes sonication, heating, grinding, etc. (Zhao et al., 2010)

6. Properties of Chitosan

The individual polysaccharides chains of chitosan (Table 1) have monomers which exhibit chiral properties with three crystal types including *α*, *β*, and *γ*, where *α-crystal* type is the most abundant in natural chitosan sources (Xia, 2003). The reactive functional groups present on monosaccharide unit are an amino/acetamido group, and hydroxyl groups (primary and secondary). The structural, physico-chemical, intra- and inter-molecular hydrogen bonds generation ability of chitosan have been correlated to these reactive functional groups. The amino group reacting with aldehyde or amide derivatives of acylating reagents allows imine formation due to its nucleophilic properties. Cationic properties of polymer are demonstrated by its ability to generate salts and its correlation with chelation, flocculation, and biological functions. The dispersal of acetyl groups along the polysaccharide chain is responsible for solubility, as well as H-bonds inter-chain interaction and acetyl group hydrophobic nature of chitosan (Younes et al., 2014)

Table 1
Mechanical and thermal properties of chitosan (Martel-Estrada et al., 2015)

S.No	Property (Unit)	Value
1	Elastic Modules (KPa)	3,790
2	Comperession Strength (KPa)	589
3	Molecular Weight (Daltons)	3,800 and 20,000
4	Deformation at Maximum Strength (%)	22.9
5	Decomposition Range (℃)	232 to 326
6	Maximum degradation rate (Tmax) (℃)	271.35 ℃

Higher viscosity of chitosan in solution form is attributed to its high molecular weight (Martel-Estrada et al., 2015). Chitosan in its polymeric form is soluble in acidic conditions, but solubility decreases in solutions of pH values above 6.3. Solubility and low viscosity in oligomeric form of chitosan is observed at neutral pH (Hirano et al., 2002; Zhang et al., 2010). Chitosan in both polymeric and oligomeric form carry positive charges, which facilitates its association with negatively charged surfaces. Several biomedical applications are possible due to this chitosan-surface binding (Kurita, 1998). Different structures of chitosan exhibit different biological properties developed through chemical modification and enzymatic hydrolysis for various prospective applications.

6.1. Biological Properties of Chitosan

Chitosan is most frequently used natural polymers while molecular weight and degree of deacetylation acts as determining factor for its properties. Molecular weight and degree of acetylation are responsible for various properties of chitosan.

6.1.1. Biocompatibility of Chitosan

Chitosan is known to have low toxicity profile in comparison to other polysaccharides. The biocompatibility and toxicity of any material depend on its ability to be biologically degraded and resultant degradation products, respectively. Chitosan degradation leads to production of non-toxic products which are easily integrated in metabolic pathways or secreted with no body accumulation/retention issues.

6.1.2. Antimicrobial Agent

Chitosan and its derivatives have wide spectrum antimicrobial properties which include activity against filamentous fungi, yeasts, and bacteria. The mode of action of chitosan involves interaction and subsequent disruption of cell membrane of microbe (Fradet et al., 1986; Lou et al., 2011; Mellegård et al., 2011; Costa et al., 2012; Lee and Je, 2013; Younes et al., 2014). Amine protonation of chitosan at certain pH leads to interaction of the positively charged ammonia (NH^{3+}) groups with cell wall causing hydrophilic and charge density changes in the cell surface. This cell permeability alteration causes the cytoplasmic constituent's leakage and ultimately death of the cell (Helander et al., 2001; Chung et al., 2003; Chung et al., 2004).

6.1.3. Hemostatic activity

Hemostasis is property of maintaining a balance of coagulation, complementary, and fibrinolytic pathways along various components of blood cells. The hemostatic activity of chitosan differ from normal mechanism observed in severe anti-coagulating conditions and abnormal activity of platelets through *in vitro* studies (Fradet et al., 1986; Subar and Klokkevold, 1992; Mankad and Odispoti, 2001). The cell membrane of blood cells (negative charge) and chitosan (positive charge) electrostatic interaction leads to agglutination of blood cells (Brandenberg et al., 1984; Rao and Sharma, 1997; Chou et al., 2003). Chitosan polymer chains are thought to be cross linked with these cells.

6.1.4. Antioxidant activity

Metabolic processes, exogenous factors and agents produce oxygen radical precursor of various degenerative diseases and aging process. These oxygen radicals react with biomolecules (proteins, carbohydrates, lipids, and DNA) leading to cell damage. The role of antioxidants is to eradicate free radicals and prevent oxidative stress in order to protect the biological system (Brasselet et al., 2019). The scavenging effect of chitosan-based compounds can be characteristic of their capacity to extract free radicals. Structural properties of chitosan, mainly amino and hydroxyl groups attached to the pyranose ring, can be attributed to radical scavenging property (Obara et al., 2003). The decline in intermolecular hydrogen bond leads to hydroxyl and amino group activation involved in radical scavenging process. Chitosan compounds work as radical locking along free alcohol and amino groups for free radical's entrapment and undergoing copulation reactions.

6.1.5. Anti-tumor activity

The antitumor property of chitosan and its derivatives is generally observed by low-molecular-weight hydrophilic chitosan, whereas oligo-chitosan is found to act as immunomodulator. Enhanced cytotoxic activities of chito-oligosaccharides against tumor are also associated with initiation of lymphocyte factor and increase in T-cell proliferation. High molecular weight of chitosan can induce death in cancer cells by neutralizing their strong charges and reducing viability in cancer cells (Rajasree and Rahate, 2013).

6.1.6. Anti-inflammatory activity

Treatment of inflammation by non-steroidal anti-inflammatory drugs acts through *cyclooxygenase-2* inhibition which leads to prostanoids production. The anti-inflammatory action mechanism of chitosan relies on ability to encourage inflammatory cells migration to healing site via *cyclooxygenase-2* inhibition pathway. As a result, huge collection of post inflammatory products and growth factors produced at healing site are reported (Zhang et al., 2010; Rajasree and Rahate, 2013).

6.1.7. Biodegradability of Chitosan

Chitosan is a polysaccharide with glycosidic bonds which are degraded by several proteases, lysozyme and chitinase enzymes (Kurita et al., 1998). Formation of non-toxic oligosaccharide of different length is noted as a result of chitosan biodegradation. (Kurita, 1998; Helander et al., 2001)

7. Modifications of Chitosan

Amine group along with primary and secondary hydroxyl represent reactive groups of chitosan where most of the modifications are carried out. Availability of these reactive groups is to mediate chemical properties and induce deviation in physical properties of chitin and chitosan as well. Modification, which could be introduction of a new group by replacing or adding to the reactive groups on chitosan backbone would generally not amend the basic structure of chitosan but improve or modify the exciting properties (Table 2). Modifications of chitosan can be categorized based on nature of functional groups introduced.

7.1. Chemical Modification of Chitosan

7.1.1. Quarternised Chitosan (QC)

The quarternisation of chitosan can be performed by introducing quaternary ammonium in chitosan chain utilizing various procedures including halo-alkylation (Rinaudo et al 1996; Rinaudo et al., 2005; Ortona et al., 2008; Luan et al., 2018). The positive charge on the chain and introduced alkyl moiety effect are responsible for improved properties of QC in comparison to the chitosan. The quaternary salts solubility is high in both acid and basic conditions rather than chitosan itself which has low solubility at physiological pH. The quaternary derivatives are mainly utilized due to their enhanced antibacterial potential where negatively charged cell

Table 2
List of modified chitosan with introduced group and improved properties (Rajasree and Rahate, 2013)

S. No	Modified chitosan	Introduced groups	Improved properties
1	Quarternised chitosan	Quaternary ammonium	Antimicrobial activity, solubility in water
2	Acyl chitosan	Carbonyl and keto groups	Hydrophobic properties
3	Thiolated chitosan	Thiol group	Muco-adhesive properties
4	Sulfated chitosan	Sulfate groups	Amphoteric properties
5	Sugar modified chitosan	Hydrophilic sugar moiety	Water solubility
6	Heterocyclic chitosan	Heterocyclic ring	Antibacterial activity
7	Cross-linked chitosan	Linking the chains together in 3D network	Improves strength and stability
8	Chitooligosaccharide (COS)	Reducing the molecular weight	Decreasing viscosity issues
9	Low molecular weight (LMW) chitosan	Reducing the molecular weight	Higher DPS
10	Phosphorylated chitosan	Phosphate	Osteo-conduction

surface acts as a favourable platform (Xia, 2003; Mohamed et al., 2013; Rajasree and Rahate, 2013).

7.1.2. *N-alkyl* chitosan

The *N-alkyl* derivative of chitosan are synthesized by reacting amino groups of chitosan with aldehydes and ketones which undergo Schiff reaction and subsequently reduce with reducing agents sodium borohydride ($NaBH_4$) or sodium cyanoborohydride ($NaBH_3CN$). Alkyl chain introduction on a modified chitosan (N-methylene phosphonic chitosan) enable both hydrophobic and hydrophilic branches to be present in its structure (Zhang and Hirano, 1995; Zong et al., 2000; Mourya and Inamdar, 2008). Introduction of alkyl group in *N-lauryl-N-methylene* phosphonic chitosan has been found to hinder hydrogen bond formation while increasing its hydrophobic properties. The amphiphilic properties increase along with surface activity which enhances its ability to act as surfactants, and it can find application in pharmaceutical and cosmetic field (Rinaudo, 2006; Zhang et al., 2010).

7.1.3. Carboxy Alkyl chitosan

Carboxy alkyl chitosan are synthesized by the reaction of chitosan with monohalocarboxylic acid in order to enhance chitosan's efficiency as absorption enhancer. This is achieved by overcoming the inadequate solubility. Absorption enhancement of carboxy alkylated derivatives at neutral pH values which are quite like those found in the intestinal tract and they can be utilized in pharmaceutical applications especially in drug-delivering systems, both controlled and sustained.

7.1.4. Acyl Chitosan

N-acyl chitosan is produced by the reaction of acyl halide or acid anhydride with chitosan in order to increase its hydrophobic character and to introduce some changes in structural features. Chitosan solubility is reported to be influenced by degree of acyl substitution and length of the chain. The solubility in water decreases with rise in chain length and degree of acyl substitution (Chen et al., 2015). The modified chitosan will experience reduced hydration and increased hydrophobic interactions which also helps in network stabilization.

7.1.5. Thiolated chitosan

The thiolated chitosan are synthesized by the reaction of various reagents with chitosan; such as glutathione (GSH) and thioglycollic acid (TGA) for introduction of thiol group. Formation of disulphide bonds by oxidation process of the immobilized thiol groups result in thiolated chitosan. Excellent in situ gelling properties are demonstrated by thiolated chitosan. Chitosan with thiol group conjugation have been found to enhance the muco-adhesive and controlled drug releasing properties (Rajasree and Rahate, 2013).

7.1.6. Sulfated chitosan

Synthesis of sulphated chitosan occurs through site specific modification of hydroxyl and amino groups with sulfate group using sulfating agents. Sulfated chitosan have structural similarities with heparin, which is a known blood anticoagulant. It is also known to give rise to antiviral, anticoagulant, and anti-sclerotic activities to chitosan (Rajasree and Rahate, 2013; Al Ghamdi et al., 2017). This modified chitosan exhibits anti-obesity activities by inhibition of anti-adipogenesis and blocking of malignant melanoma cell adhesion in human. Sulfated chitosan with anticoagulant, antimicrobial, and osteogenic activities is reported as a water-soluble anionic material.

7.1.7. Phosphorylated chitosan

Phosphorylation of chitosan occurs through reaction between methane sulfonate and phosphorous pentoxide acid. Cation-exchange properties of phosphate functional group can be attributed for various orthopaedic applications. This ensures that calcium ions associated with phosphate groups lead to initiation of calcium phosphate layer formation on polymeric implants. This can further facilitate the osteo-conduction of chitosan based implants (Qin et al., 2012; Rajasree and Rahate, 2013).

7.1.8. Cross-Linked Chitosan

Cross-linking of chitosan involves various chemical agents (glutaraldehyde, formaldehyde, tripolyphosphate, and polyaspartic acid sodium salt) for the purpose of linking the different chains together to create a multi-dimensional macromolecular chitosan. Resultant size and degradation rate of chitosan particles depend upon cross-linking preparation methods and chitosan employed in the process. Chitosan cross-linking is generally performed in two different ways, first by reacting with aldehyde compounds, such as glyoxal, formalin, or glutaraldehyde by formation of covalent bonds in acid or basic medium and secondly by the polyanions through formation of inter- and intramolecular cross-linkages (Mati-Baouche et al., 2014; Pellá et al., 2018). This could also be achieved by step wise utilization of ionic tail compounds covalently linked to enhance the mechanical properties in hydrogel formation (Rinaudo, 2006; Jătariu et al., 2013).

7.1.9. Chito-Oligosaccharide

High viscosity of chitosan due to its high molecular weight hinders its application at the commercial scale. This problem is solved by molecular weight reduction of chitosan through chemical or enzymatic hydrolysis procedures (Hsu et al., 2002; Cabrera and Van Cutsem, 2005; Delattre and Vijayalakshmi, 2009; Kasaai et al., 2013). The oligosaccharides are found to exhibit less viscous and more hydrophilic nature due to their reduced chain length. Chito-oligosaccharides with low degrees of polymerization (DPs) e.g. 20 and average molecular weight approximately 3,900 Da fall in category of less viscous and more hydrophilic chitosan. Many favourable biological effects such as infections protection, arthritis control, lowering blood cholesterol, antitumor properties, and calcium uptake improvement are among chito-oligosaccharide health benefits (Lodhi et al., 2014).

7.1.10. Low Molecular Weight (LMW) Chitosan

The Low Molecular Weight (LMW) Chitosan is also oligosaccharide with a degree of polymerization in the range between 11 up to 30. It is synthesized by depolymerization of chitosan through hydrolysis by acid and degradation by enzymes (Obaidat et al., 2010).

7.2. Enzymatic Modification of Chitosan

The environment issues surrounding reagents utilized in chemical modification initiated search for alternate methods and reagents for chitosan modification. Enzymes offer an alternate way of chitosan modification without harming the environment and humans in the process.

7.2.1. Arginine functionalized chitosan

In Arginine-functionalized chitosan, arginine groups are substituted on chitosan backbone to different degrees. High solubility of functionalized chitosan in water is due to high pKa value of the side chain guanidinium of arginine (pKa = 12.48). Chitosan functionalization with arginine is carried out through reaction with *1-ethyl-3-(3-dimethylaminopropyl)* carbodiimide hydrochloride and its derivatives which generate different functionalized chitosan. Antibacterial activities of chitosan were restrained by reduced solubility above pH 6.5 but with improved solubility, arginine-functionalized chitosan improves antibacterial properties (Tang et al., 2010).

7.2.2. Chitosan thiosemicarbazones

Thiosemicarbazone chitosan compounds are synthesized through condensation reaction with aldehydes or ketones such as phenyl-aldehyde through different methods (Qin et al., 2012). Thiosemicarbazone chitosan derivatives are well known for its antiviral, antitumor, antibacterial, and antifungal activities. Thiosemicarbazone chitosan compounds are also reported for their antifungal activity against common crop pathogen like fungi. During structure-activity relationship analysis, it was observed that thiosemicarbazone, and not the produced Schiff base, is responsible for antifungal activity of the compounds. Aromatic ring substituent present in chitosan compounds has specific effect on its antifungal properties. (Qin et al., 2012)

8. Characterization of Extracted chitinous materials and their modified forms

The extracted chitinous materials can be characterized using range of methods (Flow chart 1).

8.1. Physical features

8.1.1. Solubility

Chitin is generally insoluble in water, while 50 % uniformly deacetylated chitin improves its water solubility and partial solubility in aqueous HCl is also possible as *β-chitin* is transformed into non-reactive α-chitin form. (Pillai et al., 2009) Linear chitin or chitosan is comparatively less soluble than the branched chitin or chitosan. Additionally, introduction of sugar derivatives in branched chain increases the solubility of the molecule. (Kurita, 1998) The solubility of chitosan can be obtained by mixing a weighed quantity of sample in

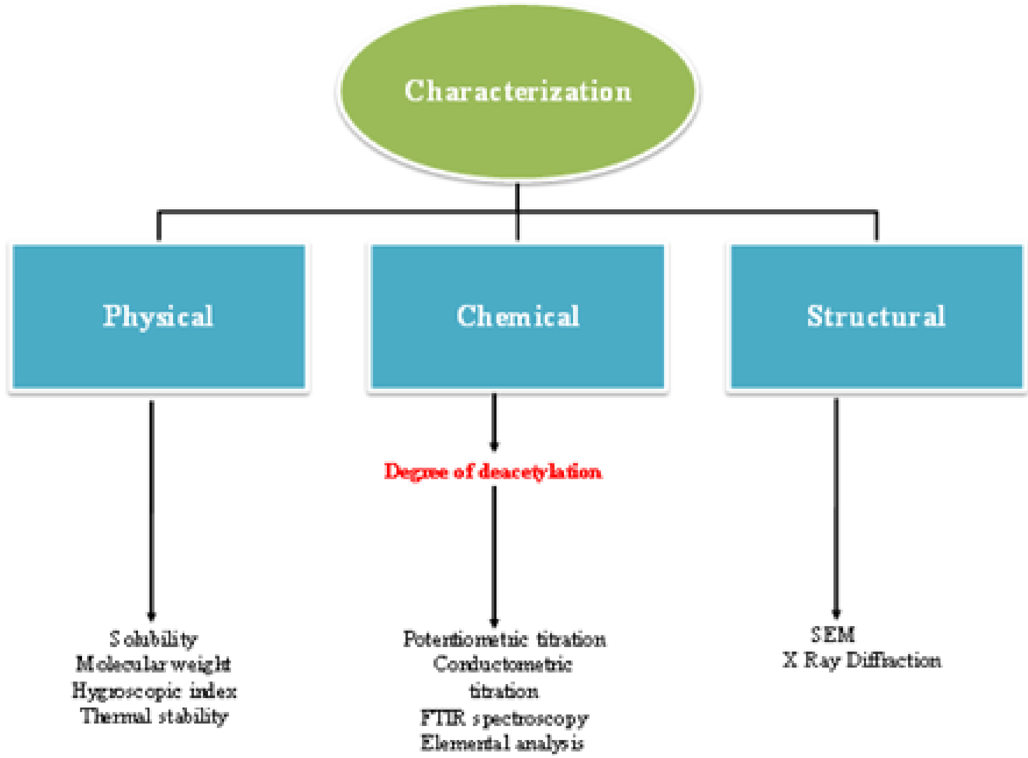

Figure 3. Various parameters included in characterization of chitin and chitosan. Different techniques involved in characterization of chitin and chitosan

measured volume of water. After a certain period, sample is filtered and undissolved part is collected, dried and weighed. From this the amount of sample solubilized could be measured. (Desbrières et al., 1996; de Queiroz Antonino et al., 2017)

8.1.2. High performance size exclusion chromatography

Size exclusion chromatography helps in determining molecular weight of samples and is extensively used for determining the mw of chitosan extracted from natural sources. The eluent that could be used is 0.2 M acetic acid / 0.1 M sodium acetate. Before running the sample in sephacryl columns, standard is plotted by using various molecular weight dextran samples. Later the samples are run in the column and eluents are analyzed using a RI detector (Kittur et al., 2002). In case of the absence of detector, the eluents could be analyzed using a colorimetric method. Here, the sample is treated with concentrated H_2SO_4 which breaks the glycosidic bond and converts pentose into furfural and hexose into hydroxymethyl furfural. When this product is treated with phenol, yellow-gold color is formed which could be estimated at 480 nm for pentose and 490 nm for hexose. From this absorbance, the concentration could be determined by plotting the values against the respective standard curves. (Nielsen, 2010)

8.1.3. Hygroscopic Index

Hygroscopic index of chitosan is the measure of its ability to absorb moisture and eventual trapping. This could be measured by keeping a weighed amount of dry sample in room conditions for 7 - 8 days after which the weight is again measured. Increase in weight shows that the sample has trapped moisture from the surrounding. Branched derivatives of chitin and chitosan tend to have a high hygroscopic index than the linear derivative of these molecules. (Kurita, 1998)

8.1.4. Thermal stability

Thermal stability of chitosan is generally analyzed by thermogravimetric approach. Here, the sample is heated at a certain temperature range and loss of weight is measured as a function of thermal stability. Pure chitin and chitosan show the initial peak at 90 - 99 °C which is due to the water molecules trapped in these samples. The second peak for chitosan appears at 303 °C, whereas for chitin it appears at 373 °C. Based on the data, it can be concluded that chitin is thermally more stable than chitosan. But few modifications of chitin or chitosan can alter their stability (Abdou et al., 2008).

8.1.5. Degree of Deacetylation

8.1.5.1. Potentiometric titration

As discussed in previous sections, deacetylation and its extent are important structural changes which govern many of the physico-chemical and biological properties of extracted chitosan, and it is important to evaluate degree of deacetylation after extraction and modification processes. Potentiometric titration is one of the important methods to evaluate degree of deacetylations. The experimental protocol involves dissolving of 0.5 g of sample in 25 ml of 0.1 M HCl whose ionic strength is maintained at 0.1 using KCl. During the next step, the sample is titrated against 0.05 M NaOH and a graph is plotted where Y-axis depicts pH and X-axis depict volume of NaOH. The graph will have two inflection corresponding to which the volume of NaOH is noted. With these values, DD % can be calculated using the formula (Abdou et al., 2008):

$$DD\ \% = \frac{1 - 161 \cdot Q}{1 + 42 \cdot Q} \ and \ Q = \frac{N \cdot \Delta V}{M} \tag{2}$$

where: $DD\ \%$ is Degree of deacetylation, ΔV is Difference in NaOH volume between two points, N is NaOH Concentration, and M is dry weight of chitosan.

8.1.5.2. Conductometric titration

Conductometric titration is an indirect way of measuring the Degree of Deacetylation (DD) in chitosan. In this technique, the amine group of chitosan is saturated with excess of concentrated acid and the remaining unreacted acid is measured by titrating it against NaOH. The titration is monitored with the help of conductometer which senses the ion concentration as a function of conductivity. A 0.5 % amount of the sample is dissolved in 0.05 mol/L of HCl. After 18 hrs of mixing in shaker, 100 ml of water is added to the above mixture and titrated against NaOH (Dos Santos et al., 2009).

The DD can be calculated using the following formula:

$$x = 20{,}319.23 \cdot \frac{C \cdot \Delta V}{42.0367 \cdot C \cdot \Delta V + W \cdot m} \tag{3}$$

where: x - Degree of Deacetylation; C - NaOH concentration; ΔV - Change in volume of NaOH, W - Solid mass fraction of Chitin.

8.1.5.3. Infrared Spectroscopy

FTIR is one of the sensitive techniques for the detection of DD. The most widely used procedure is pelleting out 1mg of sample with 100 mg of KBr such that the thickness is around 0.25 mm. Then the pellets are placed in the FTIR setup and scanned from 400 cm^{-1} to 4,000 cm^{-1} in absorbance mode. After scanning, DD % can be calculated using the formula as described in the paper (Brugnerotto et al., 2001):

$$DD\ \% = \frac{31.92 \cdot A_{1320}}{A_{1420}} - 12.20 \tag{4}$$

where: A_{1320} - Absorbance at 1320 cm^{-1}; A_{1420} - Absorbance at 1420 cm^{-1}.

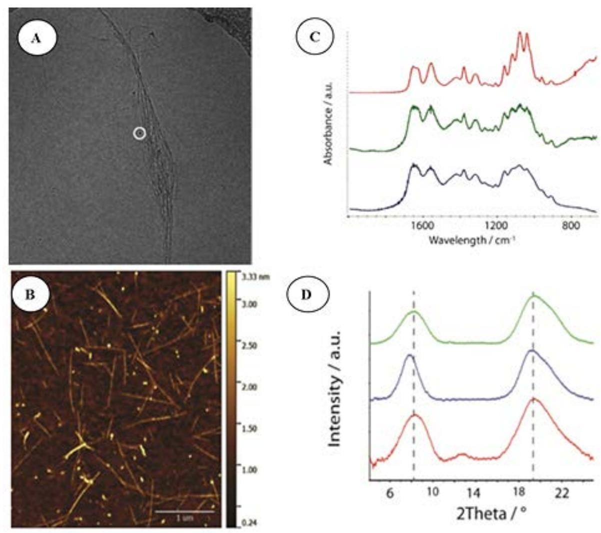

Figure 4. Characterisation of Chitin Nanofibril (CNF): (A) TEM image of β CNF; (B) AFM of β CNF; (C) FTIR spectra of β CNF; (D) XRD of β-Chitin (Blue) and β CNF (Red). (Montroni et al., 2019)

8.1.6. Elemental analysis

Using an elemental analyzer, the composition of individual atoms C, H, and N could be analyzed. Measurement of elemental composition pure chitosan with isolated chitosan determines whether the isolated sample has any other functional groups. (Yen et al., 2009) From elemental analysis data, DD % can also be calculated using the formula (Abdou et al., 2008):

$$DD\ \% = \frac{\left(6.857 - \frac{C}{N}\right)}{1.7143} \tag{5}$$

where: C - elemental composition of Carbon, N - elemental composition of Nitrogen.

8.1.7. Structural and Topographical analysis

Even though chitin isolated from any source has the same chemical composition, the topographical features may vary depending on the environment in which they were present. This makes it important to study their topographical features.

8.1.7.1. Scanning electron microscopy (SEM)

SEM is generally done to study the surface topography of the samples. For chitosan samples, SEM is performed

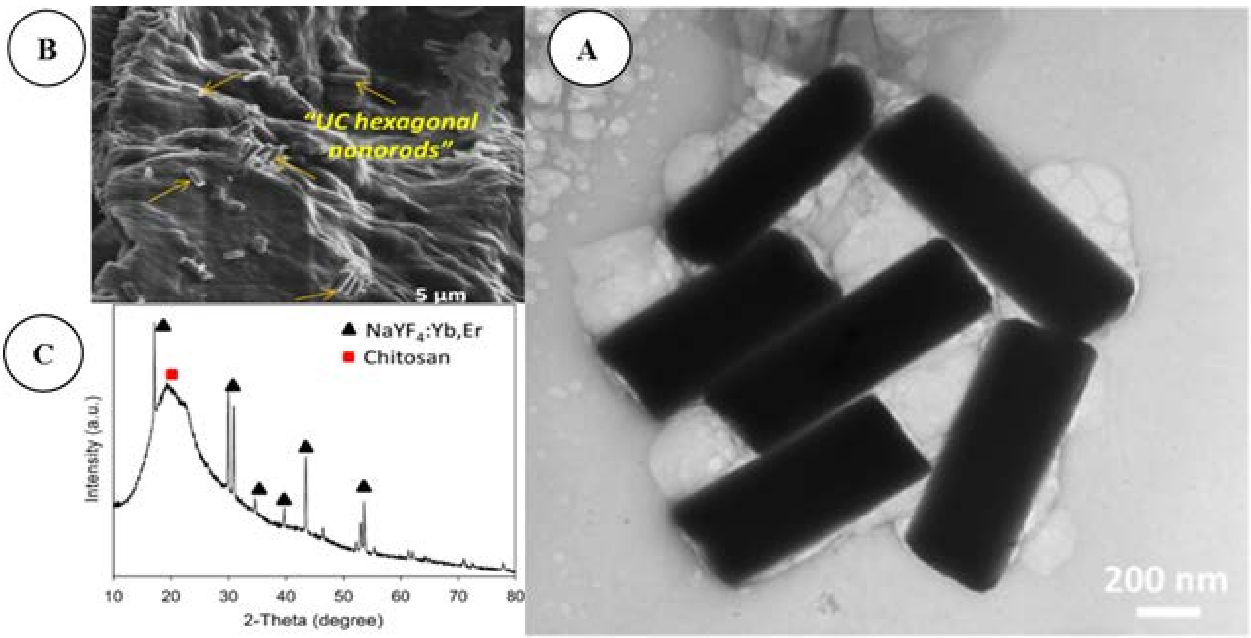

Figure 5. Characterisation of $NaYF_4$: Yb,Er /chitosan composit; (A) TEM image of aqueous dispersion; (B) SEM image of aerogel composite; (C) XRD patternof aerogel composite. (Duong et al., 2018)

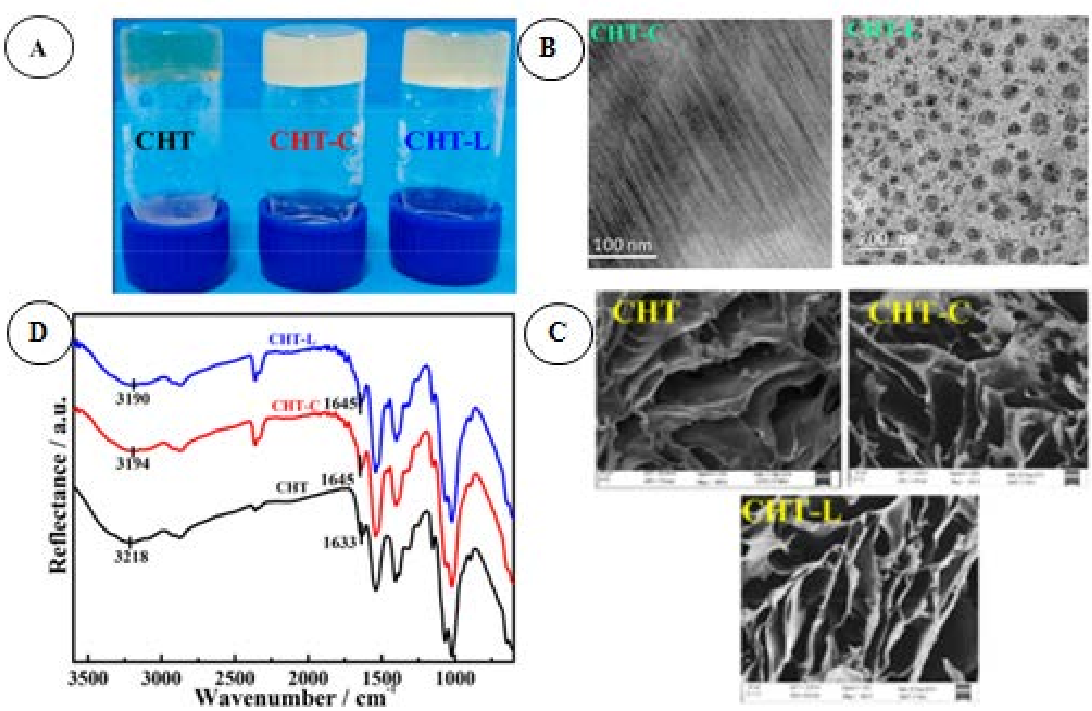

Figure 6. Characterisation of chitosan (CHT) nanohybrid in hydrogel and scaffold for bone regeneration. Nanohybrids developed with two nanofillers 30B nanoclay (CHT-C) and with layer double hydroxide (CHT-L); (A) Photographic image of CHT, CHT-C and CHT-L; (B) TEM image of CHT-C and CHT-L; (C) SEM image of CHT, CHT-C and CHT-L; (D) XRD pattern of CHT, CHT-C and CHT-L (Mahanta et al., 2019)

by making them electrically conductive. As carbon tapes are adhesive and conductive material, chitosan samples are coated with carbon tapes with additional gold sputtering, if required before performing SEM. These changes help in imaging the sample with good contrast.

8.1.7.2. Transmission Electron Microscopy (TEM)

TEM offers better spatial resolution than SEM. TEM analyzes inner structure and features, such as size, morphology on atomic scale, and surface properties. The chitin and chitosan samples used need to be thin (< 100 nm), in order to facilitate the transmission of electron beam.

8.1.7.3. Atomic Force Microscopy (AFM)

AFM offers statistical data, including size, surface area, and volume distributions. AFM helps in obtaining high resolution 3D image of chitosan. Through tip movement without any coating or damaging the sample, AFM measures the height of the chitin and chitosan samples. AFM can work effortlessly in room temperature without any vacuum environment.

8.1.7.4. X ray diffractometry

The atomic arrangements of any crystalline material including chitosan can be deduced using XRD method. By dividing the area of crystalline peaks with the total area under curve, the relative crystallinity of the sample could be analyzed. (Abdou et al., 2008) Studies show that pure chitin is more crystalline than chitosan and, hence, the stability of chitin is comparatively high. The X-ray diffractograms of the chitin and chitosan samples show two sharp diffraction peaks at 2θ of about 9 - 9.5 ° and 10 - 10.5 ° from (020) planes and 19 - 19.5 ° and 20 - 20.5 ° from (110) planes of crystalline unit cells. (Rout, 2001)

9. Biomedical Applications of Chitosan

9.1. Tissue engineering

Tissue engineering (Figure 7) is a modern advancement of biology which promises to replace, regain, or support biological functions of any damaged tissue. (Qazi et al., 2014; Ahmed et al., 2018) Different natural or artificial biomaterials have been explored for various tissue engineering applications in the form of hydrogels, composites, and bioceramics. Among naturally occurring biopolymers, chitosan is of great significance for tissue engineering applications owing to its sustainability, biocompatibility, and biodegradability. Another property of chitosan which makes it suitable for tissue engineering is that, it is easily processable in various forms like nanofibres, hydrogels, membranes, nanoparticles, scaffolds etc. (Kim et al., 2008) These different forms have their different purposes in field of tissue engineering. Some of the major areas of tissue engineering include tissue engineering, liver tissue engineering, cardiac tissue engineering, and nerve tissue engineering, where chitosan based scaffolds are widely used (Artan et al., 2010; Park and Kim, 2010; Tan et al., 2012; Kulkarni et al., 2017).

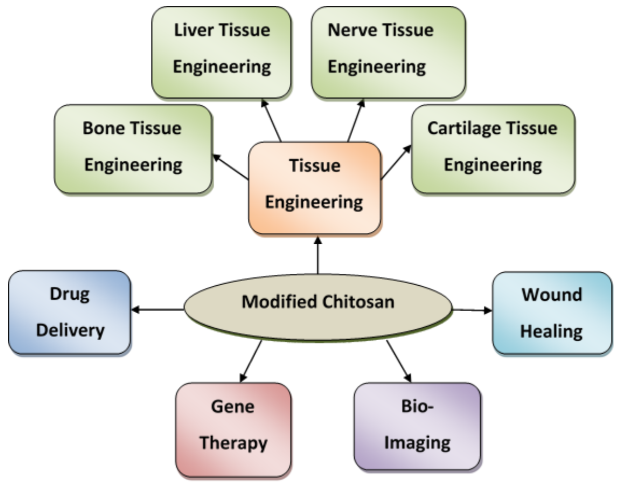

Figure 7. Block diagram showing various areas of chitosan utility. The modified chitosan with its improved properties fulfills the requirement of various biomedical applications

9.1.1. Bone tissue engineering

Bone tissue engineering is one of the most common and widely explored field of tissue engineering (Figure 8), which is concerned with creating artificial bone implants to support or replace the biological function of a damaged bone. In some clinical conditions like osteoporosis, arthritis, bone infections, or in some skeletal defects due to trauma, craniofacial surgeries etc. severe bone damages occur which cannot heal by themselves. In this condition artificial bone implants or bone tissue engineering strategies become important to regain the original functionality. The main objective of bone tissue engineering is to design a 3D scaffold that mimics ECM like environment and provide support to the newly growing bone tissue (Li et al., 2005; Saravanan et al., 2016). The scaffolds for bone tissue engineering are designed in such a manner that they promote adhesion, survival, and migration of osteogenic cells. The scaffold must also have all the physical, as well as biological factors i.e. growth factors, required for proliferation of osteoblasts. Simply put, a scaffold is devised with multiple techniques to enhance the growth of bone tissue. The modified chitosan is widely used as biomaterial for scaffolds fabrication. Some recent research studies suggest that chitosan nanofibre based 3D scaffolds are very promising for bone tissue engineering applications (Logith-Kumar et al., 2016) because, fibrous structures provide larger surface area along with high porosity and they also have desired mechanical and physical properties (Amini et al., 2012). All these factors improve the regeneration capacity of the tissue. In some other studies combination of chitosan and bioactive glass nanoparticles are used to prepare novel bone regenerating membrane. These membranes are found to have high regenerative potential (Mota et al., 2012). Hu et al. (2017) fabricated hybrid scaffolds composed of natural polysaccharides in-situ strategy. Electrospining technique is generally used for the generation of nanofibers from chitosan, which can be used along with gel-based systems like gelatin to gain desired physical properties of the scaffolds.

9.1.2. Nerve tissue engineering

Various natural and artificial materials have been used in tissue engineering field to achieve regeneration of any body tissue or organ. Some clinical conditions like diabetes mellitus induced neuropathy, nervous system diseases, peripheral nerve injury, sensory disorders etc. have great impacts on patient's health. Approximately three decades back the only solution for damage nerve repair was autograft. But insufficient donor source, tissue mismatch, graft rejection, and unsatisfactory response limited this autograft strategy for human subjects. In recent years tissue engineering approaches have allowed the use of biomaterial derived scaffolds for nerve regeneration. (Yi et al., 2019) As mentioned earlier, the basic idea behind using biopolymeric scaffold is to improvecell attachment, cell growth, and neovascularization for growth of new tissues by providing 3D porous structure. These scaffolds are designed in such a way that it should mimic the real environment of peripheral nerve matrix and should be biodegradable and non-toxic. Various in-vitro, as well as in-vivo studies, have proved that chitosan-based fibers and membranes support the survival and growth of hippocampal neurons and Schwann cells, suggesting their use in nerve tissue engineering. A recent in vivo study concluded that suturing a chitosan-based nerve scaffold into the damaged sciatic nerve of a rat model could induce a notable motor and sensory functional recovery. Chitosan based nerve implants are also proved to recover a long-distance peripheral nerve defect in diabetic rat (Mohammadi et al., 2013). Gonzalez-Perez et al. (2015) evaluated regenerative capability of chitosan tubes compared to silicone tube nerve autografts to bridge critical 15 mm nerve gap. In their study, they found that chitosan tubes had 57 % success rate, whereas regeneration failed with silicone tubes. A chitosan/polyglycolic acid nerve conduit was found to bridge and repair 30 mm long sciatic nerve gap in canine model. These chitosan/polyglycolic acid scaffolds can also repair delayed long-term peripheral nerve injury. In another study, the fabrication of an aligned cryomatrix-filled nerve guidance channel was reported. These nerve guidance channels were fabricated by 3D printing along with nerve growth factors for nerve regeneration (Singh et al., 2018). All these studies ultimately suggest that an engineered chitosan-based nerve scaffold is much better option for nerve damage treatment because it provides all the beneficial outcomes of an autograft and excludes autograft related problems.

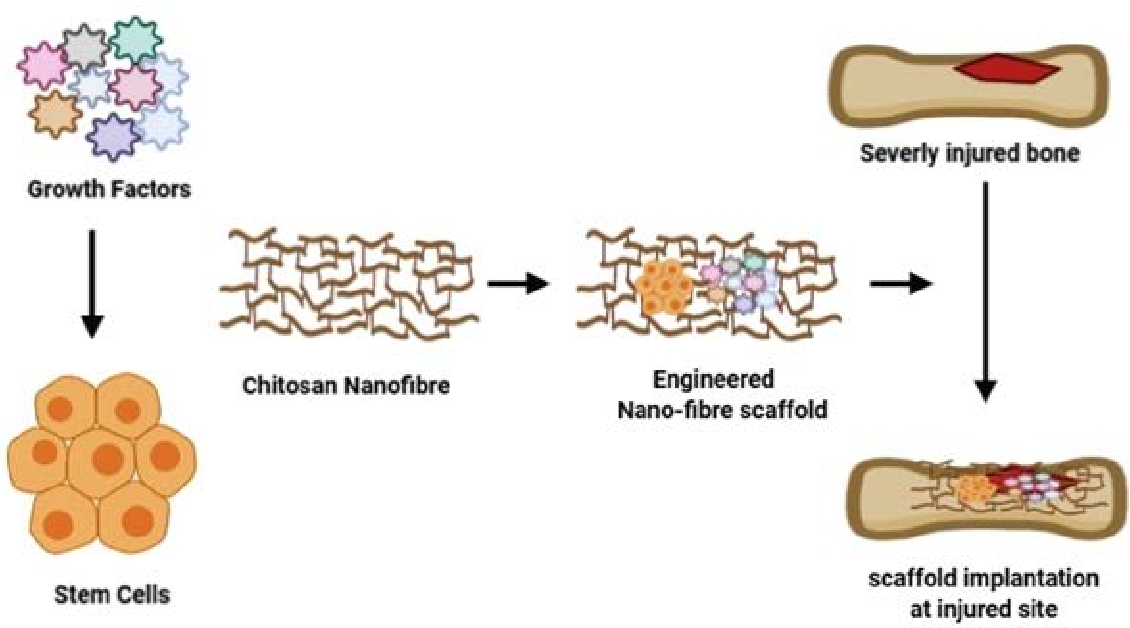

Figure 8. Schematic diagram showing bone tissue engineering strategy using chitosan nanofibre, growth factors and stem cell. The growth factors and stem cells are incorporated in the chitosan nanofibre scaffold to design nanofibre scaffold with combine benefits of better adhesion of scaffold as well as biological factors i.e. growth factors, required for proliferation of osteoblasts.

9.1.3. Cartilage tissue engineering

The treatment strategies for one of the most common age related disorder, osteoporosis, involves cartilage tissue engineering approaches. The basic idea behind this is quite similar to other branches of tissue engineering. The idea is to design a scaffold with a biomaterial incorporated with various factors that support adhesion and proliferation of cartilaginous cells at affected site and hence help the cartilage to regenerate. These scaffolds are designed in such a way that the cells adhere and increase their number, and these proliferating cells are in turn induced to show cartilaginous phenotype. The scaffold material should have some important properties like biocompatibility, elasticity, biodegradability, and non-immunogenic properties, i.e. degradation products of the material should not induce any immunogenic response in the body. By considering all these properties some naturally occurring biopolymers are suggested for this purpose that are proteinaceous in nature like collagen, keratin, fibroin, elastin, and polysaccharides such as chitosan, hyaluronan, and polyesters such as poly (hydroxybutyrate) (Alves da Silva et al., 2010). Among these materials, use of chitosan is preferred for scaffolds preparation since its structure is quite similar to many glycosaminoglycans which are found in articular cartilage (Kuo et al., 2015). In some of the recent studies, chitosan based hydrogels incorporated with TGF-β1 containing microsome have been found to be very efficient for cartilage regeneration therapy. Few years ago, infuseable chitosan hyaluronic acid hydrogel for cartilage tissue engineering was synthesized by Park et al. (2017). It was found that incorporation of Hyaluronic acid (HA) in hydrogels enhanced the proliferation and deposition of the cartilagenous extracellular matrix by the encapsulated chondrocytes. In another study, Tan et al. (2012), could synthesized infuseable biodegradable chitosan and HA based hydrogels by Schiff's base reaction of the amino groups of *N-succinyl* chitosan and the aldehyde groups of HA. The other properties of hydrogel like swelling capacity, porosity, gelation time, texture etc depended on *N-succinyl* chitosan and aldehyde HA ratio during the synthesis of the hydrogel. So, it should be considered during hydrogel preparation. In another study, tissue reconstruction efficiency of chitosan hydrogel scaffold for chondrocytes was tested in sheep models and it was revealed that chitosan hydrogels can repair cartilage defects in nearly 24 weeks (Hao et al, 2010).

9.1.4. Liver tissue engineering

In disease conditions like acute or chronic liver failure the only treatment option available is liver transplantation. But since availability of donors is very limited, the new tissue engineering based therapies are emerging. Liver tissue engineering approaches are used to regenerate damaged tissue and to replenish its original function. Structural resemblance of chitosan to glycosaminoglycans, makes it more suitable to use as a scaffold for hepatocyte culture. In some of the studies alginate-galactosylated chitosan scaffolds are proven to advance hepatocyte attachment. A basic need in liver tissue engineering is a perfect extracellular matrix for the hepatocytes to sustain high-level liver defined functions. Oxidized alginate covalently cross-linked with galactosylated chitosan is used for scaffolds preparation for liver tissue engineering. The swelling capacity and degradation rate depend on alginate concentration in chitosan scaffold. Hence alginate-chitosan ratio is very important during preparation. *In-vitro* biocompatibility research demonstrate that hepatocytes grow on scaffold with a distinctive spheroidal morphology which is further found very promising for liver tissue regeneration. It is also good for the improvement of liver defined functions and improvement of successful bioartificial liver devices through tissue engineering. There are two strategies, Exogenous and Endogeneous stem cell therapy, that can be followed for liver regeneration therapy An integrated hybrid poly(N-isopropylacrylamide)-chitosan cryogel based bioreactor was previously developed that showed better efficacy in terms of detoxification as well as synthetic functions than the conventional bioartificial liver device setup (Damania et al., 2017). Stem cell based therapies are also being used to achieve liver regeneration after injury. Two most common stem cell based therapies are endogenous stem cell therapy and exogenous stem cell therapy. (Yu et al., 2017)

9.2. Role of bio-extracted chitosan in wound healing

Wound healing is a complex process in which torn or injured site of skin and damaged tissue repair themselves. The overall process of wound healing involves a highly regulated pathway of biochemical reactions in order to repair the damage. In general, if wound follows the limited healing stages in a defined time period it is called **Acute wound** and if it takes too long to heal then it is considered as **Chronic wound**. Chronic wounds are difficult to heal and require treatment or wound dressing strategies in such a way that it provides a proper platform to accelerate the epithelialization, angiogenesis, and also reduce complications such as scar formation, discomfort, pain etc. Several studies have shown that moist local environment near the wound significantly enhances re-epithelialization.

9.2.1. Hydrogel based wound dressing material

Apart from various wound dressing materials available in the market the hydrogel-based systems are found more promising for wound management. Hydrogel systems can absorb high amount of water due to the presence of hydrophilic groups which results in gel formation. Since hydrogels imbibe water and fluids, they eliminate the chances of wound desiccation. In some cases moist environment may delay the healing process, so the choice of dressing material depends on the type of wound.

Chitosan is found to be very promising for making hydrogel based wound dressing materials because of its superior properties like biocompatibility, biodegradability, antimicrobial activity, and the most important, optimum moisture absorption capacity. To achieve higher benefits, chitosan can be incorporated with polyvinyl alcohol (PVA), Sodium alginate, and Pluronics (polymers of polyethylene oxide). These are all hydrophilic, nontoxic, and FDA approved materials for biomedical applications. Several studies have proved that use of multiple polymers instead of a single polymer improves required mechanical and physical properties of hydrogel film. A recent study has revealed that hydrogel based wound healing materials prepared by combinations of different polymers show optimum swellability, flexibility, and crosslinking ability. These properties together accelerate wound healing by means of re-epithelialization (Park et al., 2009). In these properties, polymeric formulation of chitosan and pluronic are foundto be more suitable. The development of multifunctional collagen and chitosan hydrogel show enhanced wound healing and promote hemostatic ability. Different mass ratios are used to compare their antibacterial property apart from self-healing properties (Figure 9). (Ding et al., 2020)

9.2.2. Chitosan Nanofiber scaffold based wound dressing material

Nanofibrous wound dressing materials have many potential advantages over conventional dressings because of their higher surface area and microporous structure. These nanofibre based systems mimic a native extracellular matrix environment which attracts fibroblasts to the epidermal layer of wound sites. These fibroblasts secrete growth factors, cytokines, collagen, and ECM components which accelerate angiogenesis and re-epithelialization at wound site. This ECM like property occurs due to Chitosan, which has a structure similar to GAGs (glycosaminoglycans) of native ECM. Apart from these, microporous structures of chitosan materials absorb exudates from wound and allow proper ventilation at wound site for faster damage repairing. As discussed previously, some important properties of chitosan, such as biocompatibility, biodegradability, and the most important antimicrobial activity, make it more suitable for burn and wound healing. It is proven that nanofibers of chitosan accelerate the wound healing. Various hydrophilic polymers (PVA, PEO, PVP) are also used to prepare blend form of chitosan nanofibre scaffolds. The purpose of adding these hydrophilic polymers is to reduce viscosity and enhance electrospinability of the solution which is required to prepare nanofibers (Sweeney et al., 2014).

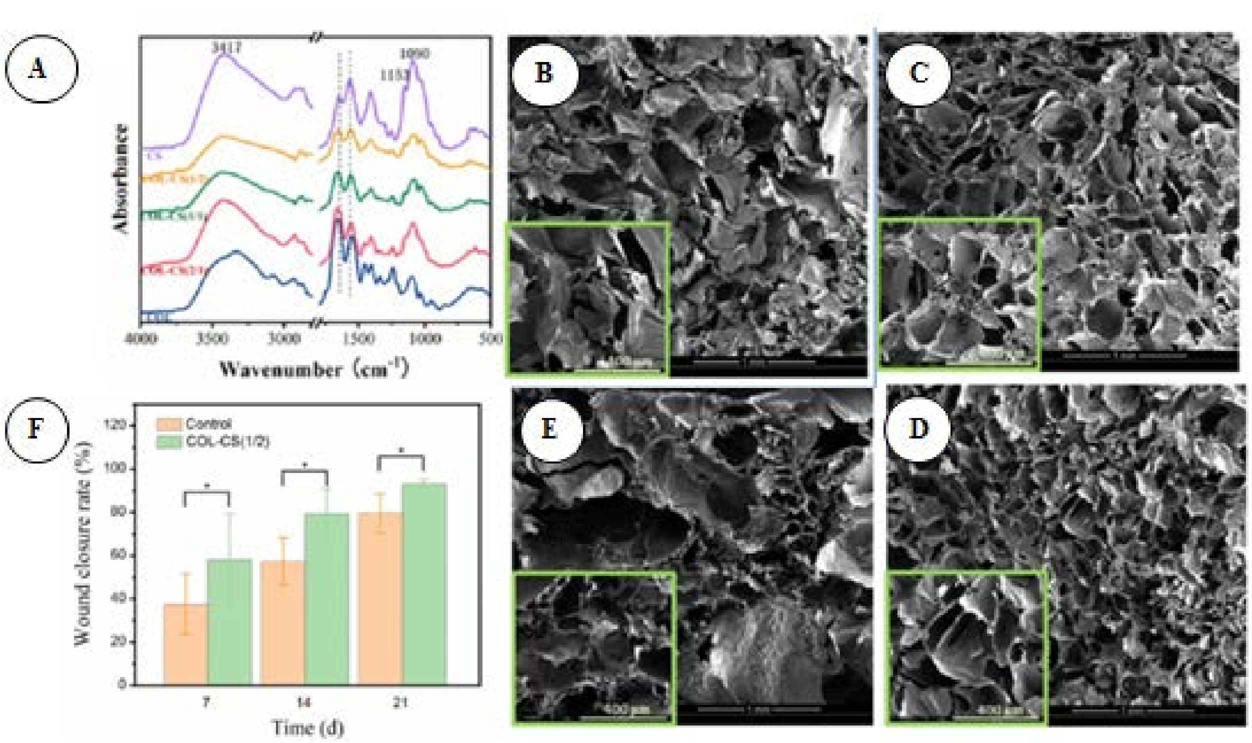

Figure 9. Collagen-Chitosan based self-healing hydrogel (COL-CS): (A) FTIR spectra of collagen, COL-CS (different mass ratio), Chitosan. SEM image of (B) Chitosan; (C) COL-CS (2/1); (D) COL-CS (1/1); (E) COL-CS (1/2); (F) Quantification of wound closing area. (Ding et al., 2020)

Oleoyl chitosan and gelatin in weight ratios (100:0, 90:10, 75:25) is utilized through electrospinning to develop nanofiber scaffolds (mat). The developed nanofibres have improved adhesion and proliferation in presence of human amniotic membrane derived stem cells (Figure 10). Additionally, some chemical crosslinkers are also used in system which improve optimum hydrophobicity of the material so that it can maintain its native structure in aqueous medium. Recent studies on canine model have proved that nanofibrous mat of chitosan along with Poly (caprolactone) and Poly (vinyl alcohol) in an optimum mass ratio of 1:2:1.5, enhances complete epithelialization with amount of new glands regenerations with complete regeneration of epidermal cell. (Datta et al., 2017)

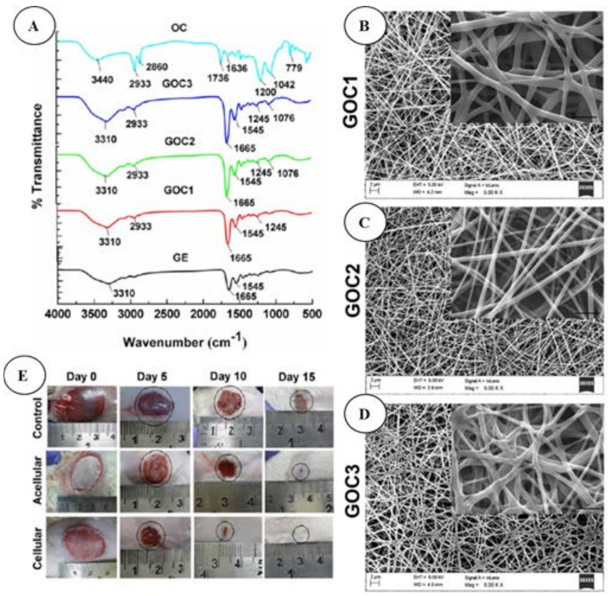

Figure 10. Nanofibre scaffold based on Oleoyl-Chitosan/ Gelatin (GOC): (A) FTIR spectra of OC, GOC (GOC1, GOC2, and GOC3), and Chitosan. SEM image of GOC: (B) GOC1; (C) GOC2; (D) GOC3; (E) Optical image of wound healing in control, acellular and cellular. (Datta et al., 2017)

9.3. Chitosan for drug delivery

Due to its favorable physiochemical properties such as biological degradability and biocompatibility, chitosan is preferentially used for targeted and localized drug delivery. Chitosan plays a role of most important polysaccharide for controlled drug delivery for targeted treatment of many diseases including cancers. The use of chitosan for cancer treatment overrules the side effects of non-targeted and non-specific therapies which are painful, low effective, and high cost. After oral administration nanopolymers possess easy absorption in gastrointestinal tract due to which these nanopolymers have proved as the potential candidates as drug carrier in cancer treatments. Chitosan nanopolymers prepared as a nano-carrier for delivering drug in chemotherapy by mixing it with hydrophobic surface coating agents and making it stable nano-carrier till it reached target tumor site (Shanmuganathan et al., 2019). In 2018, Samrot et al. (2018) utilized crab shell derived chitosan for synthesis of polymeric nano particles, and these nano particles were further encapsulated with hydrophobic curcumin and analyzed for drug delivery *in vitro*. In 2019, host-guest chemistry-driven supramolecular chitosan nanogels were reported that were stimuli responsive thus allowing selective drug release in specific cancer cells or disease sites (Ding et al., 2019). In order to find the most suitable drug delivery system, the capacity of chitosan nanocomposite systems containing *N-doped* graphene or *P-doped* graphene nanoparticles for delivery of anticancer drug ifosfamide was examined by means of molecular dynamics simulations (Shariatinia and Mazloom-Jalali, 2019). Moreover, chitosan aerogels as drug delivery vehicles provided highly porous network, considerably large specific surface area, and polycationic feature and, thus, offered improved drug bioavailability and drug loading capacity (Wei et al., 2020). López-Iglesias et al. (2019) utilized vancomycin loaded chitosan aerogels which could devote to treat and prevent infections at the wound site (López-Iglesias et al., 2019).

Chitosan is proved to be versatile and so it could be used for application in controlled release of drug formulations for treating acute and chronic wounds. Chitosan hydrogels, being nontoxic, stable, biocompatible, and biodegradable, are mainly used for pharmaceutical and biomedical purposes such as drug delivery in wound healing (Hamedi et al., 2018).

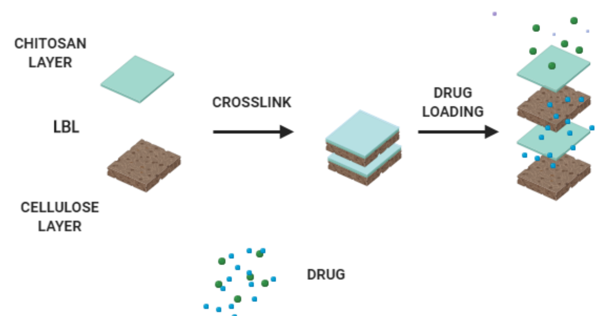

Figure 11. Multilayer nanofilm of Cellulose and Chitosan prepared by Layer by Layer Method (LBL) and chemically cross-linked to improve the inner structure of resultant multilayer. The inner structure of multilayer is responsible for drug loading and release

Chitosan as a promising matrix proved effective in treatment of chronic wounds like burns and diabetic ulcers too (Hamedi et al., 2018). Chitosan is being used for delivering local anesthetics which helps in controlled release of anesthetics to minimize side effect of uncontrolled and nonspecific anesthetics delivery like benzocaine, lidocaine, and tertacaine (Di Martino et al., 2019). Polyoxometalates - an antitumor agent inhibits the action of SOX_2 and it is effective in reducing the risk of metastasis - is found to be harmful for normal cells due to its cytotoxicity. Development of chitosan nanogels loaded with covalently cross-linked Wells-Dawson type phosphomolybdate $[P_2Mo_{18}O_{62}]^{6-}$ was identified with great capability for targeted delivery of polyoxometalates in a noncytotoxic drug release manner at physiological pH (Pérez-Álvarez et al., 2019).

9.4. Chitosan in Gene Delivery

Chitosan is being widely used in gene therapies. The most critical step for gene therapy is release of gene sequence in intracellular space. Although viruses are proved to be the promising and useful gene delivery vectors, but limited to be used for gene delivery due to immunogenicity, toxicity, and sometimes uptake issues, a requirement for some non-viral vectors has risen. As nucleic acid and bio membrane are negatively charged, chitosan being natural cationic polysaccharide can interact with both and is one of the most versatile non-viral vectors.

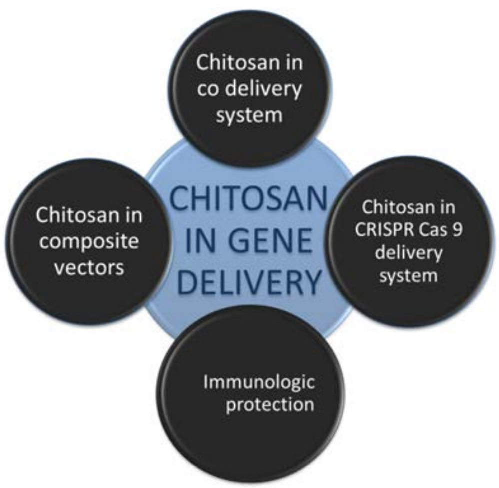

Figure 12. Various aspects of using chitosan in gene delivery

Native chitosan is modified to improve its ability as a vector (Fig. 12). One of these modifications includes improving solubility as chitosan is poorly soluble at neutral and alkaline pH conditions. Chitosan can also be modified with small molecules such as mannose and mannosylated chitosan is effective in gene therapies (Chuan et al., 2019). For escaping endosomal fusion chitosan is modified with PEI for improving buffering capability that leads to the proton sponge effect that depends on chitosan's buffering capacity (Chuan et al., 2019). Chitosan as successful non-viral vector has many applications in gene therapy systems including CRISPR/Cas9 delivery systems (Li et al., 2015). In basic research CRISPR/Cas9 is widely applied for therapeutic and genome regulation purpose but use of viral vectors have safety issues and have packaging limitations. Material science and nanotechnology improve by applying synthetic vectors in place of viral vectors which are capable of targeting cells and tissues with optimal physiological conditions (Li et al., 2015). Chitosan encapsulated with red fluorescent protein proved to be successful vector for delivery of Cas9 ribonucleoproteins into the nucleus. Assembly of RFD-Chitosan Cas9 and ssDNA enters the cells through the endocytosis followed by entry to the nucleus to elicit homology directed repair (HDR). RFP-Chitosan can serve as the fluorescent probe to track the delivery and facilitate tacking of transfer efficiency (Qiao, 2019). Because of easy transport and adhesive properties in gut, oral delivery of chitosan-DNA construct has been demonstrated in several studies that is nearly impossible by using viral vectors. These oral administrations can be achieved when chitosan and DNA form a stable nanoparticle and enter gut. The endocytosis exhibits immunologic protection against several food allergies such as peanut allergy (Roy et al., 1999).

9.5. Chitosan in Bioimaging

In past several years, chitosan has been used for bioimaging. Chitosan derived nanostructures exhibit strong fluorescence upon excitation with ultraviolet. These polysaccharide-based nanostructures also proved to be stable in terms of photo-bleaching and some metal ions can have quenching effect on it. At a concentration of 10 mg/ml chitosan nanostructures show least cytotoxicity (Zu et al., 2016). In one study, immediately after the previous one, chitosan-based core shells proved to be used for near infrared imaging under NIR-I radiation. In this study, chitosan based nanospheres emitted bright NIR fluorescence that could be used in both in-vivo and in-vitro in tracing nanospheres (Tan et al., 2017). In one of the most recent study, the authors have developed $Fe_3O_4@SiO_2$ coated chitosan for bioimaging in Zebrafish model. GdOF: Ce^{3+}, Tb^{3+} were used as luminophore, which efficiently emitted green when excited at 280 nm. They have tried these nanomaterials in zebrafish and there was no acute cytotoxicity reported that proved biocompatibility (Khan et al., 2019).

10. Future Prospective

Chitin and chitosan, due to their diverse applications are estimated to make important advances in the field of biomedical. These materials can act as improvised prospects for health industry, financial growth, and development due to broad spectrum availability in biowaste, optimized methods of extraction and green methods of modifications. Extraction of chitin and chitosan from wastes produced by different industries is an economical and green process as no harmful chemicals are used. However, these processes are time-consuming and governed by various fermentation parameters like pH, temperature, growth span, etc. Microbial contamination is also one of the important factors that hinder the production of good quality of such materials. Additionally, one of the important drawbacks of isolating chitin and chitosan from different wastes is that there is no single step process for the same which might reduce the overall yield of the product. These concerns are tuning future prospects of this area of interest as further optimization of all sustainable methods of interventions. It majorly includes extraction, modification, and application with high yield without losing gained properties of being better polymeric materials for various biomedical applications. Some of these can be achieved by minimizing harsh conditions of extraction from biowastes, less consumption, and high re-usability of enzymes used during chemical modifications and better and broad range tunability of physico-chemical properties for different applications.

Chitosan and their derivatives are one of the most promising biomaterials in the industry. These chitinous derivatives are used in various processes like wound healing, tissue engineering, drug delivery, etc. Since these biomaterials are produced by natural assets, these are more viable than synthetic materials produced chemically right now. Additionally, these biomaterials can find their way in the biomedical industry, as a scaffold for tissue engineering. This will reduce the cost of second surgery for removing the scaffold. Chitin and chitosan are highly biocompatible compared to the other synthetic polymers used. Therefore, they prevent any treatment for sensibility and rejection from the implants/materials formed from them. There are still some challenges associated to these materials regarding chemical and mechanical properties of these materials. The nanomaterials formed using these materials can have acute or chronic toxic effects on the body which is the primary challenge faced. Therefore, chitin and chitosan derived biomaterials are needed in tissue engineering implants which can simultaneously decrease the immune responses.

11. Conclusion

Chitin and its derivatives are the as important biopolymers present in the nature as cellulose. They can be isolated abundantly from seafood wastes like crustaceans, shrimps, etc. However, alternative methods for isolating chitin and its derivatives from industries like silkworm, honeybee industry, etc. have paved a way to produce chitin and chitosan from the industrial wastes. The isolation from these wastes can be done chemically or biologically. The chemical methods are used conventionally. However, the use of chemical methods can lead to change in the physical and chemical characteristics of the sample. It can change other properties like molecular size, charge, etc. Therefore, biological methods are making their way into production/isolation. They minimize the irregularities which are obtained during chemical isolation of chitin and chitosan. Isolation of these polymers from the waste materials is a way to maintain ecological balance as well economical preferences. Chitin and chitosan are very versatile polymers which help in exploring diverse models of industrial function. Chitin and chitosan can also be used for improving the quality of existing polymers and implants. Thus, it is important to not only optimize the extraction of chitinaceous materials from natural sources but also their post extraction modifications, characterizations and plausible applications in various field. These understandings will motivate more industrial scale utilization of biowaste for production of chitinaceous materials and their use in developing economical products for human welfare in sustainable manner. Accordingly, this review has discussed various methods of extracting chitinaceous materials from biological sources, characterizations, modifications, and use in various industries. It also reveals the benefits associated with chitinaceous materials extracted from natural sources by biological methods and modified by bioenzymatic processes. They have also been correlated with areas of their application ranging from tissue engineering to drug and gene delivery. This review will surely be helpful to readers in providing overall information about sustainable availability and applicability of chitinaceous materials.

Acknowledgement

Authors would like to acknowledge the support from Indian Institute of Technology Kanpur and course BSE613A (2019-2020) in preparation of this manuscript. Authors declare no conflict of interest.

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Ekstrakcija hitina i hitozana iz biološkog otpada za primenu u biomedicini

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INFORMACIJE O RADU

Primljen 17 jun 2020
Prihvaćen 30 septembar 2020

Pregledni rad

Ključne reči:
Biološki otpad
Hitin
Hitozan
Zarastanje rana
Davanje lekova
Inženjering tkiva

I Z V O D

Biomaterijali su dizajnirani tako da omoguće interakciju sa biološkim sistemima prilikom zarastanja rana, regeneracije tkiva, davanja lekova i kao mehanička potpora kako bi se poboljšali trenutni terapijski rezultati. Primena biomaterijala u zdravstvu se neprestano povećava u praksi i samim tim biomaterijal postaje biokompatibilniji i manje toksičan u fiziološkim uslovima. Primena ovih materijala je povezana sa poboljšanjem terapijskih ishoda kod ljudi, međutim, doziranje leka potrebnog za uspešno lečenje bolesti se obično razlikuje kod svake osobe i oslanja se na iskustvo lekara konsultanta. To može dovesti do ljudskih grešaka prilikom odlučivanja o dozi leka, neprilagođenih implanata ili uklanjanja istih što bi za posledicu imalo negativan ili manje pozitivan efekat. Personalizovana medicina i uređaji ukazuju na to da lek treba prilagoditi pacijentu na osnovu različitih karakteristika, kao što su pol, starost, genetika i način života. Ovakav personalizovani medicinski pristup podrazumeva različite vrste lekova, metode aktivacije, nano-sklopove, biomedicinske uređaje i slično. Među tim pristupima, personalizovani biomedicinski uređaji su postali popularni nakon pojave tehnologije 3D štampe, tehnike koja se koristi za pravljenje implantata koji se mogu prilagoditi svakom pacijentu uz minimalni trošak, za kraći vremenski period i sa velikom preciznošću. Personalizovana biomedicina takođe podrazumeva i dizajniranje leka koji bi udovoljio potrebi pojedinca sa minimalnim neželjenim efektima. U ovom radu je predstavljen pregled različitih aspekata prilagođavanja biomedicinskih sredstava poput terapijskih biomolekula, nanomedicine, implantata i uklonjenih implantata. Ovaj sveobuhvatni pregled literature ukazuje da bi upotreba tehnologije 3D štampe u proizvodnji personalizovanih biorazgradivih implantanata koji otpuštaju lekove mogla predstavljati dobro terapijsko rešenje za čitav niz medicinskih stanja.

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Recycling and Sustainable Development

RSD

Online ISSN 2560-3132
Print ISSN 1820-7480

Potential impact of COVID-19 pandemic lockdown on environmental parameters

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A R T I C L E I N F O

Received 11 August 2020
Accepted 15 October 2020

Research article

Keywords:
Air quality
COVID-19
Environment
Erbil City
Lockdown
Pollution

A B S T R A C T

COVID-19 had an impact on the daily life, human activities, various sectors, and the environment. Accordingly, the aim of this research was to examine the effect of the COVID-19 pandemic lockdown (COVID-19 PL) on the environmental parameters. The studied environmental parameters were solid waste, noise, air, water, wastewater (WW), soil and green areas, natural environment and resources, light pollution, radiation pollution, energy and others. The main environmental issues were divided into seventy sub-parameters. Results revealed that COVID-19 PL increased a number of parameters, such as domestic and hospital wastes, noise at home, aquatic life and water quality, domestic WW amount, green areas, animal and birds movement, natural energy, rodents etc.; while, it decreased several factors for instance commercial/industrial solid waste, traffic and outdoor noises, air pollution and particles, water contamination, WW production, cutting trees and hunting, fuel extraction and mining, artificial light and radiation, fuel combustion, tourist etc. Alternatively, some parameters, such as black water, natural radiation, and normal lighting remained as before COVID-19 PL. Positive, nil, and negative impacts of the parameters on the environment due to COVID-19 PL were 81.43 %, 5.71 %, and 12.86 %, respectively. Positive impacts of the COVID-19 PL on the environment were greater than negative influences and lockdown was regarded as a respiration of the natural environment. Currently, prediction of seasonal impact on spreading COVID-19 is difficult.

1. Introduction

Coronaviruses started in the 1930s when it was found in domesticated chickens. In humans, it was initially documented in the 1960s. This virus contains severe respiratory tract infections (Khoshnaw et al., 2020). The coronavirus disease is a pandemic happening by an outbreak that occurred in late 2019 (COVID-19), caused by the coronavirus-2 virus of the severe respiratory syndrome (SARSCoV-2) (Ibarra-Vega, 2020). The COVID-19 was first recognized in December 2019 in Wuhan-China between a group of patients that were presented with an unknown system of viral pneumonia with joint history of visiting the Huanan seafood market (Ibarra-Vega, 2020; Peeri et al., 2020). In the past, six main pandemic and epidemic outbreaks cleaned the planet from 2000 to 2019, namely Severe Acute Respiratory Syndrome (SARS) between 2002 and 2004, H1N1 influenza in 2009, Middle East respiratory syndrome (MERS) from 2012 to 2020, the West-African Ebola virus epidemic during 2013 to 2016, the Zika fever from 2015 to 2016, and Avian influenza between 2008

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doi: 10.5937/ror2001049A

and 2014. None of these attained the spatial level and the extensive impacts that the new COVID-19 did (Cheval et al., 2020).

To date of 28 July 2020 (10:34 am), there have been 16,301,736 confirmed cases of COVID-19, containing 650,069 deaths, according to WHO reports (https://covid19.who.int/). The COVID-19 caused acute international socioeconomic confusion, such as postponement and termination of politics, sports, religious, and cultural activities (Sarwar et al., 2020).

Human activities, industrialization, climate change, global warming, population increase, technologies, crisis etc. caused effect on the environment and enhanced the environmental contamination. On the other hand, natural phenomenon, such as volcanos, dust storm, fires, deforestation, flood etc. increased environmental pollution as well. COVID-19 PL affected the environmental issues via decreasing some factors and increasing others.

A number of researches regarding the effect of COVID-19 PL on air quality (Kerimray et al., 2020; Li et al., 2020), air pollution (He et al., 2020), air quality and water quality in India (Lokhandwala and Gautam, 2020), air quality and meteorological variables (Xu et al., 2020), air quality, noise, beaches, and recycling (Zambrano-Monserrate et al., 2020), surface water quality (Yunus et al., 2020), air and water quality (Cheval et al., 2020), GIS (Sarwar et al., 2020), solid waste (Ouhsine et al., 2020), stone quarrying and crushing areas (Mandal and Pal, 2020), solid waste and beach evaluation in Ecuador (Ormaza-Gonzales and Castro-Rodas, 2020) were published. But to date, there is no published research on the effect of COVID-19 PL on the environmental parameters like solid waste, noise, air, water, wastewater (WW), soil and green areas, natural environment and resources, light pollution, radiation pollution, energy and others.

Therefore, the objective of this research was to examine the impact of COVID-19 PL on the mentioned environmental parameters. To date, this kind of study is not available in the extant literature.

2. Materials and methods

The current work focused on the studying of the impact of COVID-19 PL on the environmental factors. Environmental issues comprise of numerous parameters such as solid waste, noise, air, water, WW, soil and green areas, natural environment and resources, light pollution, radiation pollution, energy and others. Each parameter was divided into sub-parameters. For this purpose, seventy environmental factors were studied during COVID-19 PL. The environmental limits were chosen and tested based on the experience, visiting site, and the references (Chadderton 2004; Wang et al., 2010; Aziz and Ali, 2018; Aziz et al., 2019; Rangwala, 2019). Assessment for the parameters were conducted. Effect of COVID-19 PL on the environmental issues were labeled as increasing (+1), remained as before COVID-19 PL (0), or decreasing (-1). Additionally, positive, nil, and negative influences of the factors on the environment were studied. The current work focused on the Erbil City, Kurdistan Region (KR)-Iraq environment and the global environment as well. Data were collected from Erbil City and the sources. Tabulated data were compared to the published works. Partial lockdown and closing of some areas such as schools, universities, car shows, beauty salons etc. in Erbil City, KR-Iraq started at the end of February 2020. While, full lockdowns on 14 March 2020 to 23 April 2020, 24 to 26 May 2020, 1 to 3 June 2020 and 1 to 4 July 2020 were executed in Erbil City, Figure 1. For the periods 23 April 2020 and till 28 July 2020, partially lockdowns with application of various scenarios were performed.

a)

b)

Figure 1. Erbil City centre before (a) and during (b) COVID-19 PL

3. Results and Discussions

The details of the COVID-19 PL effect on the environmental issues such as solid waste, noise, and air are illustrated in Table 1 and Figure 2.

3.1. Solid Waste

Commonly the quantity of municipal solid waste (MSW) deceased during COVID-19 PL due to stopping and delaying activities such industrial, commercial, institutional etc. Lockdown caused decreasing of industrial, commercial, demolition, and construction etc.

Table 1
Impact of COVID-19 on Environmental Parameters-Part I

No.	Parameters	Details	During COVID-19 lockdown			Impact
			Decreased	Remained as before	Increased	
1		Municipal Solid Waste	-1			Positive
2		Hospital Waste			1	Negative
3		Hazardous waste	-1			Positive
4		Domestic waste			1	Negative
5		Commercial Waste	-1			Positive
6	Solid waste	Industrial Waste	-1			Positive
7		Construction and demolition	-1			Positive
8		Gardens and park waste	-1			Positive
9		Recyclable Materials	-1			Positive
10		Agricultural waste	-1			Positive
11		Mini and super markets			1	Negative
12		Activities waste	-1			Positive
13		Traffic noise	-1			Positive
14		Institutional noise	-1			Positive
15		Commercial areas	-1			Positive
16		Industrial areas	-1			Positive
17		Gardens and public areas	-1			Positive
18	Noise	Homes			1	Negative
19		Aircraft noise	-1			Positive
20		Worship areas	-1			Positive
21		Construction and demolition	-1			Positive
22		Mini and super markets			1	Negative
23		Stadium and public activities	-1			Positive
24		Underground/Tube	-1			Positive
25		Air pollution	-1			Positive
26		Ozone problems	-1			Positive
27		CO and CO_2	-1			Positive
28	Air	Oxygen			1	Positive
29		Dust storm	-1			Positive
30		Greenhouse gasses	-1			Positive
31		Particles	-1			Positive
32		Nasty odour	-1			Positive

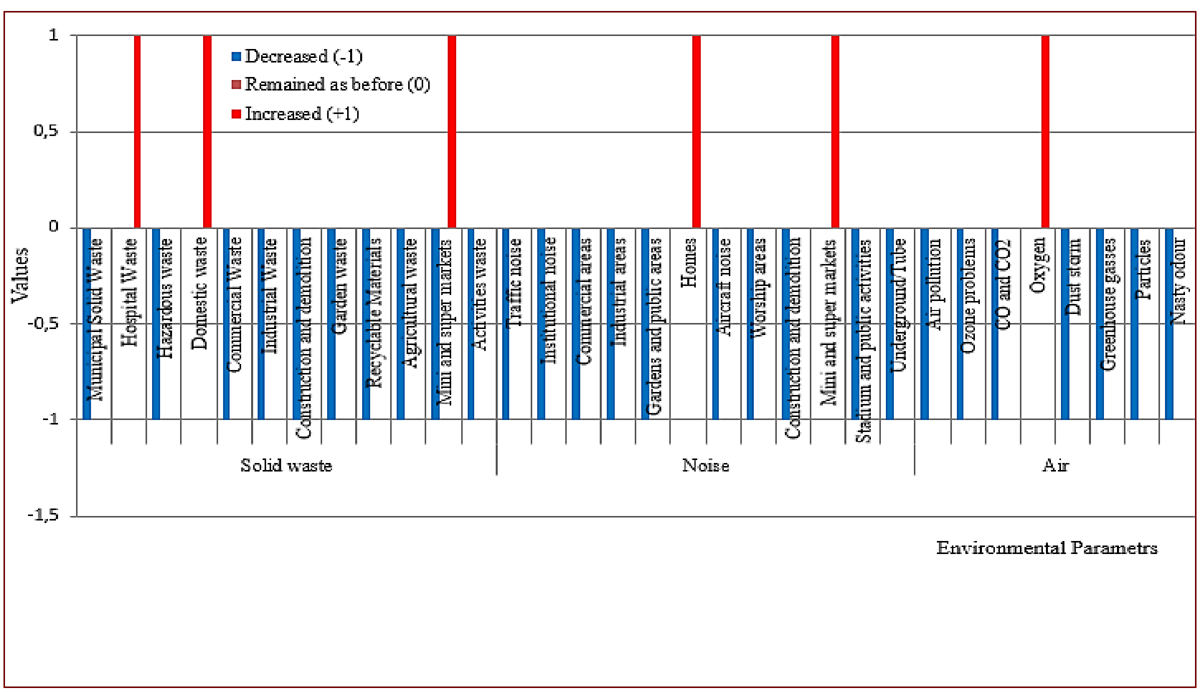


Figure 2. Impact of COVID-19 PL on Environmental Parameters - Part I

wastes, Table 1 and Figure 2. Researchers conducted a study on the MSW in Khenifra and Tighassaline-Morocco. The authors documented that the amount of MSW in Khenifra City in March 2019 and March 2020 were 2,572 tons and 2,456 tons, respectively. While, in Tighassaline City quantities of MSW for the same period were 136 tons and 126 tons, respectively. The research confirmed that COVID-19 PL resulted in deceasing amount of MSW (Ouhsine et al., 2020). In another research, authors reported that COVID-19 PL caused reduction in recycling solid waste (Zambrano-Monserrate et al., 2020). Ormaza-González and Castro-Rodas (2020) stated that amount of garbage and plastic decreased during COVID-19 PL at Salinas and Manta beaches in Ecuador.

Additionally, the beaches improved during the quarantine. On the other hand, hospital wastes increased during the lockdown due to increasing of patient numbers in the hospitals. People stayed at home and bought daily requirements in the markets, this led to enhancing of domestic and local market wastes in Erbil City. Published data confirmed solid waste information in Table 1 and Figure 2.

3.2. Noise Pollution

Noise pollution sources are traffic, aircraft, machines, conversation, sounds etc. (Aziz, 2012). COVID-19 PL causes closing of markets, industrial areas, institutional, sport areas, worship places etc. Lock downs resulted in decreasing noise at commercial, industrial, worship, and sport areas. In addition, it reduced traffic, aircraft, underground, machine noises as well (Table 1 and Figure 2).

Erbil-Kirkuk Main Road, Erbil-Iraq before and during COVID-19 PL is illustrated in Figure 3. This street is very crowded, especially during daily hours, because a number of Ministries (Ministry of higher education and Scientific Research, Communication and Transportation), Directorates (Water Resources, Agriculture, Zanco Bank, Central Library, Zanco Hospital, Heart Hospital, Traffic Police, and Transportation), Presidency of universities (Salahaddin University-Erbil, and Erbil Polytechnic University), colleges (Education, Arts, Science, and Engineering), and Institutes (Erbil Technical Institute, and Erbil Administration Institute), hotels, student hostels, stadium, car shows, restaurants, fuel stations, shops, markets etc. are located on this street, Figure 3.

Traffic noise pollution on this road was 58 to 85 dB before COVID-19 PL, and air craft noise was 73 dB before lockdown (Aziz, 2008; Aziz et al., 2012).

It is clear quarantine decreased traffic, aircraft and other noises in Erbil City. A number of researchers focused on the effect of COVID-19 PL on some environmental factors (for instance noise pollution) in China, USA, Italy, and Spain. They stated that the lockdown caused reduction in environmental noise (Zambrano-Monserrate et al., 2020). At stone quarrying and crushing areas in India, noise level before and during COVID-19 PL were 85 dBA and 65 dBA, respectively (Mandal and Pal, 2020). The results confirm the noise information in Table 1 and Figure 2. Alternatively, people staying at home increased noise pollution at home and at residential areas.

a) 27 August 2019

b) 5 April 2020

Figure 3. Erbil-Kirkuk main road before (a) and during (b) COVID-19 PL

3.3. Air pollution

Air quality index (AQI) and concentrations for PM $_{2.5}$, PM $_{10}$, NO_2, and O_3 in Erbil City before and throughout COVID-19 PL are shown in Figure 4 and Table 2. It is clear that generally lockdowns decreased AQI and concentration of $PM_{2.5}$, PM_{10}, NO_2, and O_3 and improved air quality in Erbil City. Kerimray et al. (2020) stated that COVID-19 PL decreased concentration of air pollutants such as PM $_{2.5}$, NO_2, and CO in Almaty, Kazakhstan. Cheval et al. (2020) outlined that COVID-19 PL enhanced air quality in the urban areas. At stone quarrying and crushing areas in India, PM_{10} before and during COVID-19 PL were 89 - 278 μg/m^3 and 50 - 60 μg/m^3 (Mandal and Pal, 2020). In a research conducted in Yangtze River Delta (YRD) Region - China, authors reported that COVID-19 PL reduced SO_2, NO_x, PM $_{2.5}$, and VOCs emissions by approximately 16 - 26 %, 29 - 47 %, 27 - 46 %, and 37 - 57 % throughout Level I and Level II response periods, respectively (Li et al., 2020).

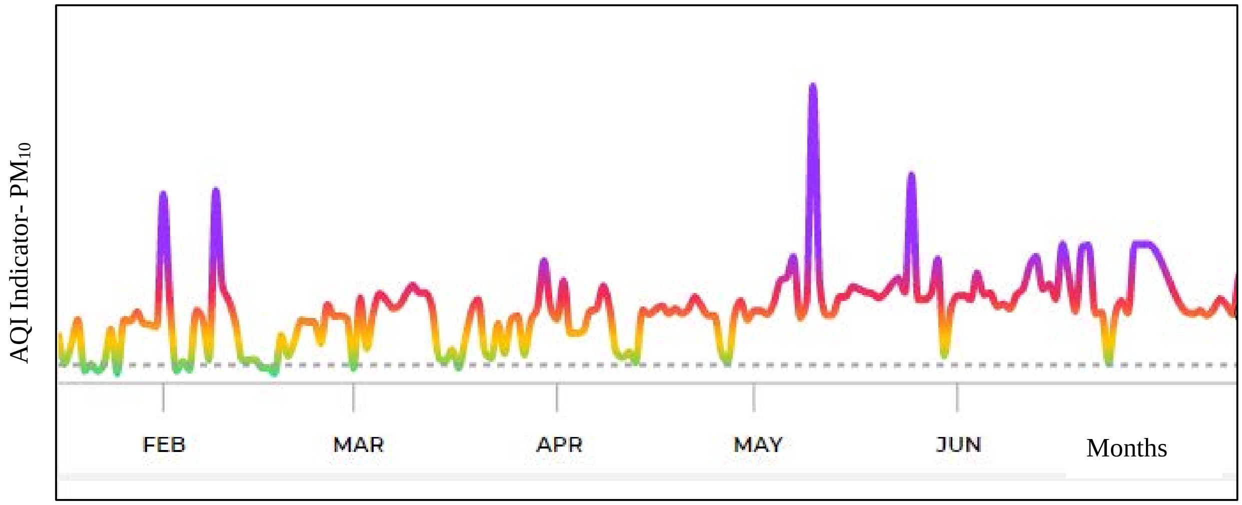


Figure 4. Air quality index in Erbil City before and during COVID-19 PL
(Air quality in Erbil, https://air.plumelabs.com/air-quality-in-erbil-oPs)

Table 2
Air quality parameters in Erbil City before and during COVID-19 PL (Air quality in Erbil, https://air.plumelabs.com/air-quality-in-erbil-oPs)

Date	AQI				Concentration (μg/m^3)				AQI Assessment	Description
	$PM_{2.5}$	PM_{10}	NO_2	O_3	$PM_{2.5}$	PM_{10}	NO_2	O_3		
17 January 2020	32	27	21	6	16	27	41	15	Average	Before Lock.
1 February 2020	103	285	7	25	55	377	13	56	Air apocalypse	Before Lock.
15 February 2020	11	14	30	7	5	14	54	17	Average	Before Lock.
1 March 2002	25	30	15	19	13	31	30	47	Average	Partially Lock.
15 March 2020	46	42	32	6	23	42	56	16	Poor	Full Lock.
1 April 2020	58	115	15	20	30	104	29	49	Very poor	Full Lock.
15 April 2020	52	117	25	4	26	106	45	10	Very poor	Full Lock.
1 May 2020	58	118	20	5	29	108	40	13	Very poor	Partially Lock.
15 May 2020	89	148	33	3	45	156	58	7	Very poor	Partially Lock.
1 June 2020	64	139	55	1	32	144	90	2	Very poor	Full Lock.
15 June 2020	62	157	30	8	31	171	53	20	Dire	Partially Lock.
1 July 2020	89	200	19	24	44	240	37	57	Extreme	Full Lock.
15 July 2020	61	164	44	4	31	183	71	10	Dire	Partially Lock.

A research carried out in locked downs cities in China, authors documented that the AQI in the locked down cities was reduced by 19.84 points ($PM_{2.5}$ down by 14.07 μg/m^3) (He et al., 2020). Reports from all over the world recognized that after COVID-19 PL, air quality improved and environment was promising (Lokhandwala and Gautam, 2020). Authors outlined that COVID-19 PL caused air quality improvement and reduction of greenhouse gases in China, USA, Italy, and Spain (Zambrano-Monserrate et al., 2020). Table 1 presented that air quality parameters reduced during quarantine. Improvement of green areas and reduction of air pollutants led to enhance the oxygen ratio. Published and collected data on air confirm the information in Table 1 and Figure 2.

3.4. Water

Influences of the COVID-19 PL on the quantity and quality of water are presented in Table 3 and Figure 5. Yunus et al. (2020) reported that COVID-19 caused improvement of surface water quality in the Vembanad Lake, the longest freshwater lake in India. Cheval et al. (2020) reported that COVID-19 PL improved water quality in the urban areas. During lockdown at stone quarrying and crushing areas in India, adjacent river water was qualitatively enhanced due to stoppage of dust release to the river.

Total dissolved solids level in river water neighboring to crushing unit decreased by nearly two times (Mandal and Pal, 2020). Documents from all over the world are specifying that after COVID-19 PL, water quality in rivers improved and nature showed signs of recovery (Lokhandwala and Gautam, 2020).

Lockdown caused decreasing of water consumption in commercial, industrial, institutional, tourist, worship etc. areas. Additionally, stopping and postponing the activities led to decreasing pollutants in the water sources and enhancing water quality.

Quarantine minimized hunting which led to the increase of fish and other aquatic lives. Published works support the ideas in the Table 3 and Figure 5.

Table 3
Impact of COVID-19 on Environmental Parameters-Part II

No.	Parameters	Details	During COVID-19 lockdown			Impact
			Decreased	Remained as before	Increased	
1	Water	Water sources improvement			1	Positive
2		Water consumption	-1			Positive
3		Precipitation			1	Positive
4		Aquatic life improvement			1	Positive
5		Water quality			1	Positive
6	Wastewater (WW)	Municipal WW quantity	-1			Positive
7		Residential WW quantity			1	Negative
8		Black water quantity		0		Nil
9		Yellow water quantity		0		Nil
10		Grey water quantity	-1			Positive
11		Industrial WW quantity	-1			Positive
12		Commercial WW quantity	-1			Positive
13		WW quality			1	Positive
14	Soil and green areas	Soil contamination	-1			Positive
15		Green areas			1	Positive
16		Erosion	-1			Positive
17	Natural Environment	Fire occurrence	-1			Positive
18		Biodiversity			1	Positive
19		Animal movement			1	Positive
20		Bird movement			1	Positive
21		Natural environment improvement			1	Positive
22		Cutting trees and grass	-1			Positive
23		Hunting	-1			Positive
24		Flood			1	Negative
25	Natural Resources	Oil Extraction	-1			Positive
26		Mining	-1			Positive
27		Quarry	-1			Positive
28	Light Pollution	Natural		0		Nil
29		Artificial	-1			Positive
30	Radiation Pollution	Natural		0		Nil
31		Artificial	-1			Positive
32	Energy	Fuel Combustion	-1			Positive
33		Natural fuel source			1	Negative
34		Biogas			1	Positive
35	Others	Rodents			1	Negative
36		Pesticides	-1			Positive
37		Composting			1	Positive
38		Tourist and picnic	-1			Positive

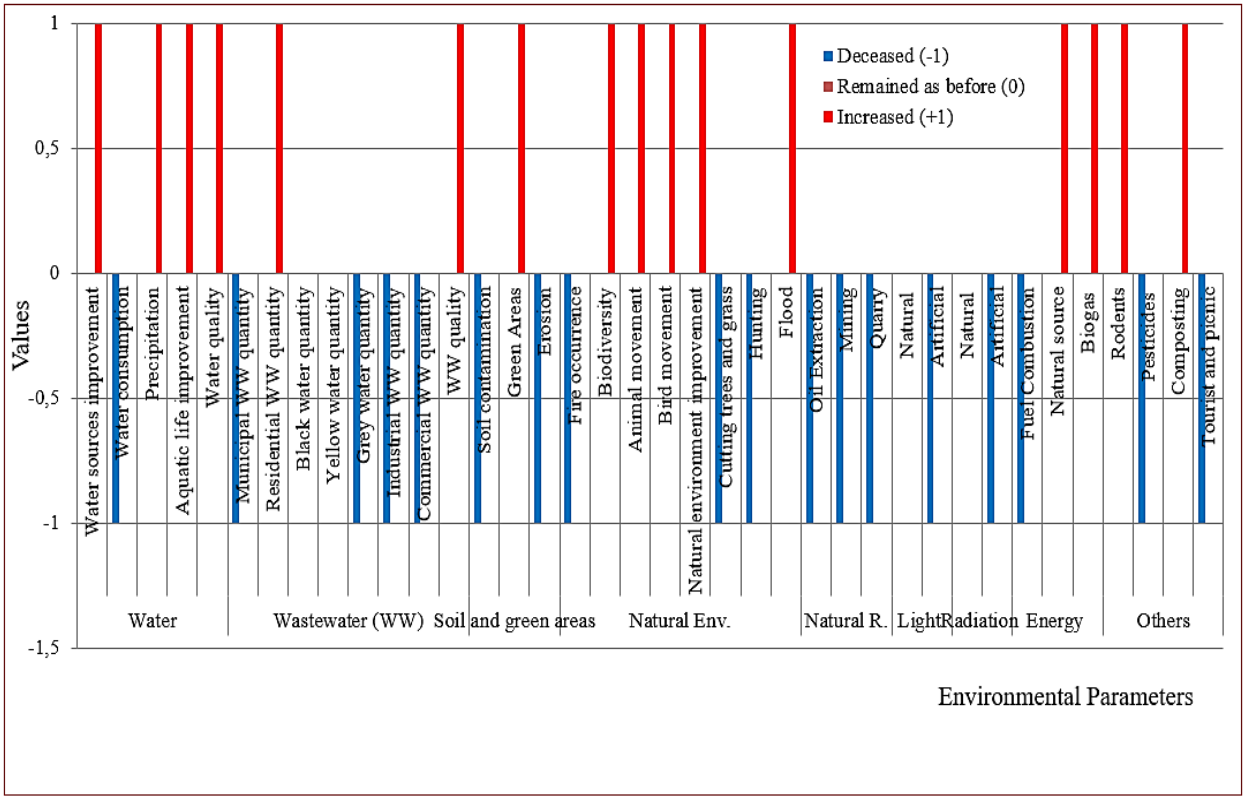


Figure 5. Impact of COVID-19 on Environmental Parameters-Part I

3.5. WW (Wastewater)

Effect of COVID-19 PL on the WW are shown in Table 3 and Figure 5. Of course, lockdown of commercial, industrial, institutional, tourist, worship and other areas caused deceasing of municipal WW (MWW) amount. Alternatively, black water and yellow water (urine) remain as before. Staying at homes led to increase of domestic WW quantity. Hospital WW discharge increased due to rising number of patients and other activities in the hospitals and labs. Industrial and commercial WWs decreased due to limitation of the industrial works. MWW characteristics improved and the amount of water pollutants reduced due to stopping and delaying activities in several sectors.

3.6. Soil and Green Areas

COVID-19 PL caused restriction of the human activities and commonly eliminated tourist; this led to increase of green areas and decreasing soil contamination, Figure 6. When green areas enhanced, corrosion decrease gradually and enhance oxygen ratio and berating.

Details of influence of COVID-19 PL on soil and green areas are shown in Table 3 and Figure 5.

a) Zanco Q., Erbil City, 2 April 2020

b) Hujran area, Shaqlawa District, Erbil City, 27 April 2020

Figure 6. Green areas in Erbil City during COVID-19

3.7. Natural Environment and Natural Resources

Natural environment and natural resources were influenced by COVID-19 PL, Table 3 and Figure 5. COVID-19 PL can be regarded as a breathing of the natural environment and decreased contaminants, Figures 1, 3, and 6. Fire occurrence, hunting, and cutting of the trees were eliminated (or minimized) throughout COVID-19 PL due to control of human activities. Movement of animals and birds became more frequent, Figures 1 and 7. Oil extraction, mining, and quarry works were limited and it resulted in decreasing of environmental pollution.

Figure 7. Staying of animals inside Zanco Q.-Erbil City during COVID-19PL (27 March 2020)

3.8. Light and Radiation Pollutions

COVID-19 PL reduced artificial light and radiation pollutions, Table 3 and Figure 5. While, natural lighting and radiation normally remained as before COVID-19 PL. Throughout COVID-19 PL most of the commercial, industrial, institutional, worship, and other sectors were closed and this led to decreasing the light pollution.

3.9. Energy

Lockdown by COVID-19 resulted in decreasing of fuel consumption, Table 3 and Figure 5. On the other hand, the use of natural fuel sources and biogas commonly remained as before or increased, especially in the countryside areas.

3.10. Others

Lastly, COVID-19 PL increased some issues such as rodents and composting. While, it decreased others like pesticides and tourist, Table 3 and Figure 5. Due to control of human activities during COVID-19, appearance and movement of rodents and animals were more frequent than before the lockdown.

The use of pesticides decreased because of the lockdown as well. Instead, staying at home led to increasing of composting process by some people. Till 17 July 2020, tourist areas and public parks in Erbil City, KR-Iraq were generally closed.

Of course, closing of tourist zones and gardens directed to decreasing of environmental pollution such as noise, water, light and air and caused a decrease of solid waste.

3.11. Positive, nil, and negative impacts

Impacts of the environmental parameters on the environment are illustrated in Table 4.

Positive, nil, and negative impacts of the environmental issues on the environment during COVID-19 PL were detected. As a result, positive, nil, and negative effects of the parameters on the environment caused by COVID-19 PL were 81.43 %, 5.71 %, and 12.86 %, respectively.

Cheval et al. (2020) stated that COVID19-PL led to negative impacts on the environment such as shoreline contamination due to the disposal of sanitary consumables. Beside of the great threats and losing many things during COVID-19 PL, it served the environment in several directions and it regarded as a respiration of the environment.

Table 4
Positive, nil and negative impacts of the parameters on the environment

No.	Parameters	Positive	Nil	Negative	Total
1	Solid waste	9		3	12
2	Noise	10		2	12
3	Air	8			8
4	Water	5			5
5	WW	5	2	1	8
6	Soil and Green Areas	3			3
7	Natural Environment	7		1	8
8	Natural Resources	3			3
9	Light Pollution	1	1		2
10	Radiation Pollution	1	1		2
11	Energy	2		1	3
12	Others	3		1	4
Total		57	4	9	70
Ratio (%)		81.43	5.71	12.86	100

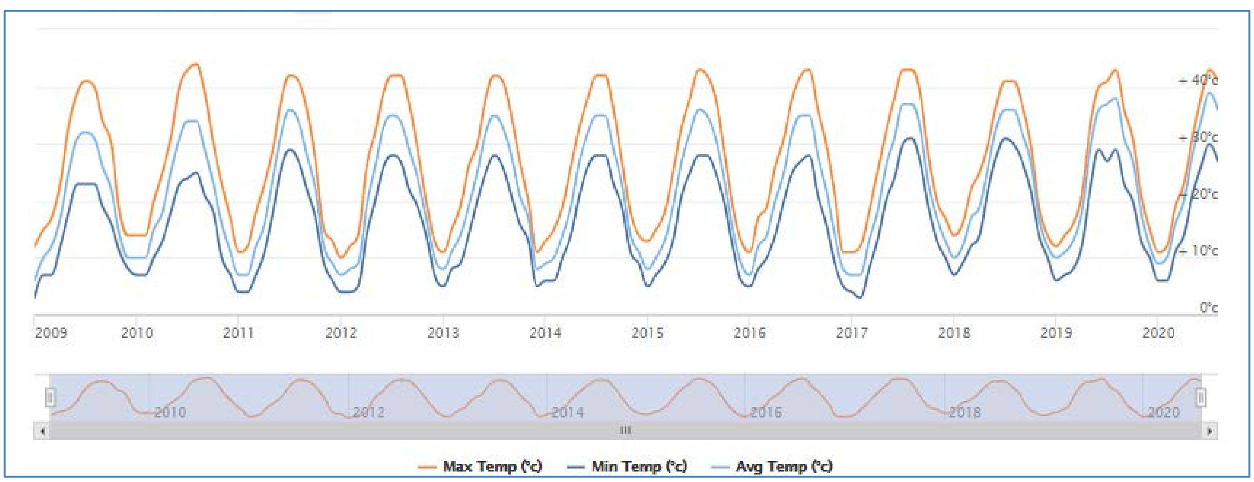
3.12. Living organisms and prediction for COVID-19 Spreading

A number of factors, such as temperature, humidity etc., affect the living organisms (McEldowney and Fletcher, 1988; Rickard and Boulding, 2015). Variations of temperature, cloud and humidity, pressure, rainfall amount and rain days, and wind in Erbil City are shown in Figures 8. It can be seen from Figure 8 that temperature, and cloud and humidity commonly remained as before COVID-19, while, pressure decreased in 2020. Additionally, amount of rainfall and wind speed generally increased during COVID-19. In 2020, pressure, rainfall, and wind varied in Erbil City and they had an impact on living organisms. Sufficient historical data is essential for forecasting of COVID-19 spreading in different seasons. At the same time, no prediction is definite as the future infrequently repeats itself in the same way as the past. Furthermore, estimates are affected by the dependability of the information, vested benefits, and what variables are being forecasted.

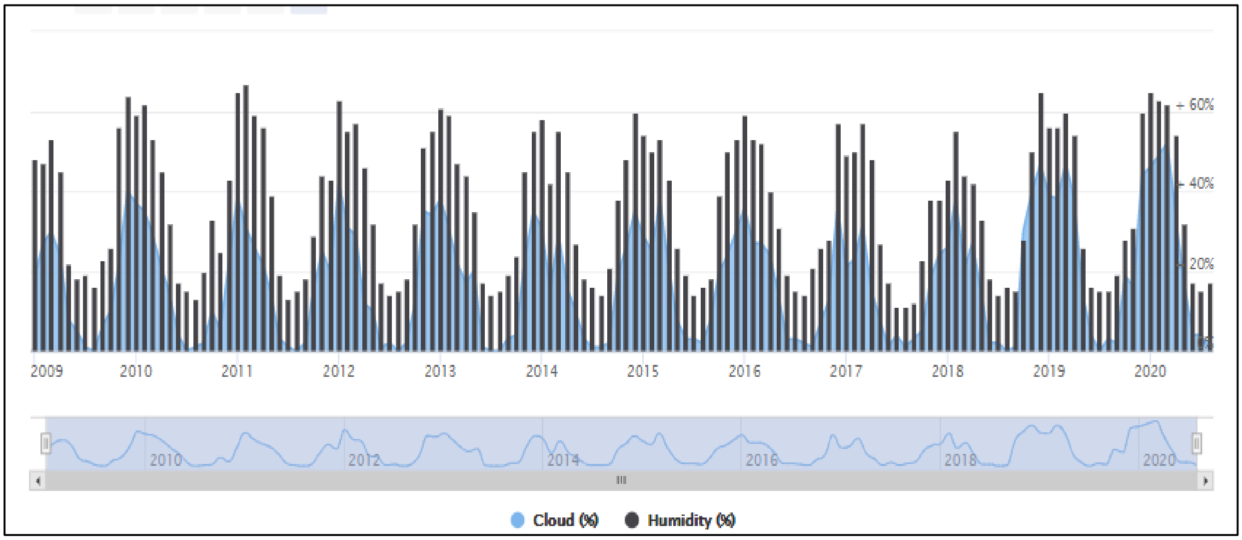
Correspondingly, psychological parameters play an important role in how people observe and respond to the risk from the disease and the anxiety that it may influence them personally (Petropoulos and Makridakis, 2020).

Additionally, Gupta et al. (2020) reported that the influence of weather on COVID-19 spread is poorly unstated. Limited published works have claimed that warm weather can probably decrease the global pandemic.

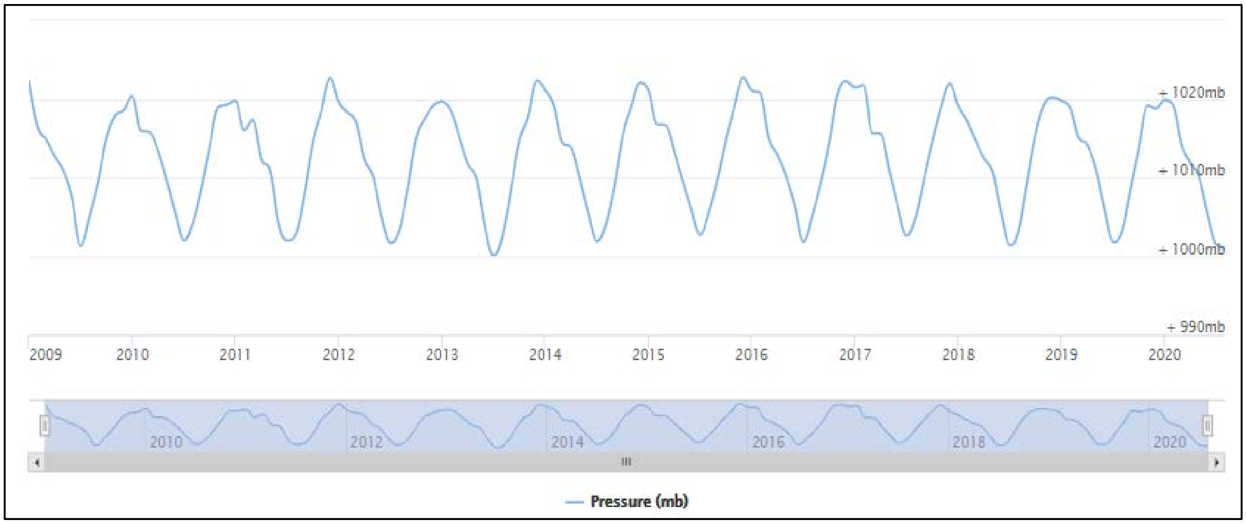
Mecenas et al. (2020) concluded that warm and wet climates seem to decrease the spread of COVID-19. The confidence of the confirmation generated was classified as low. Though, only temperature and humidity parameters could not clarify most of the changeability in disease transmission.



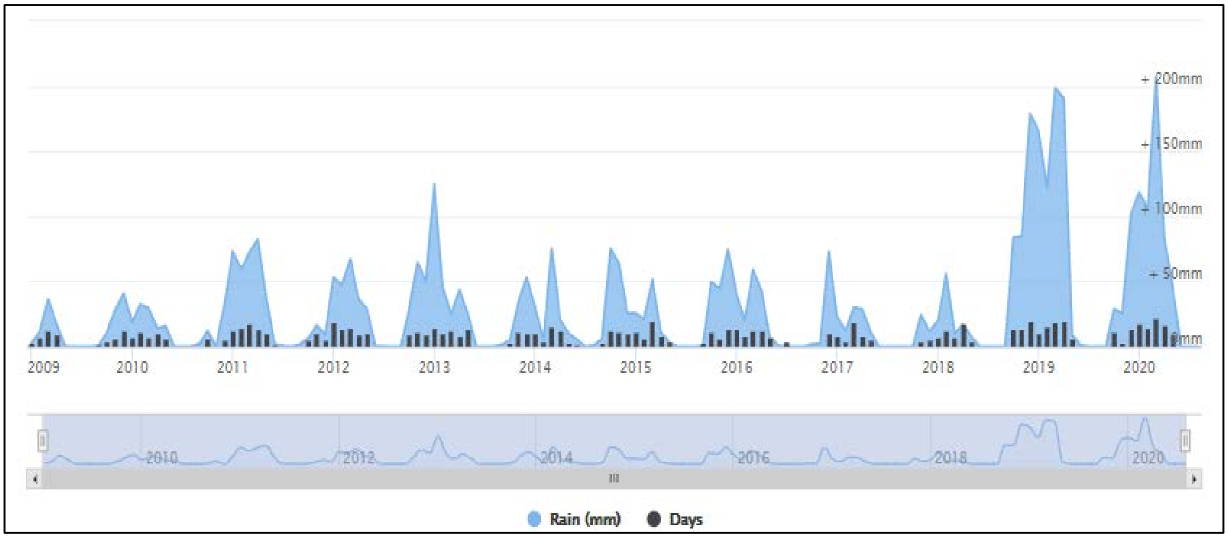
a)


60%
40%
20%
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
Cloud (%)
Humidity (%)

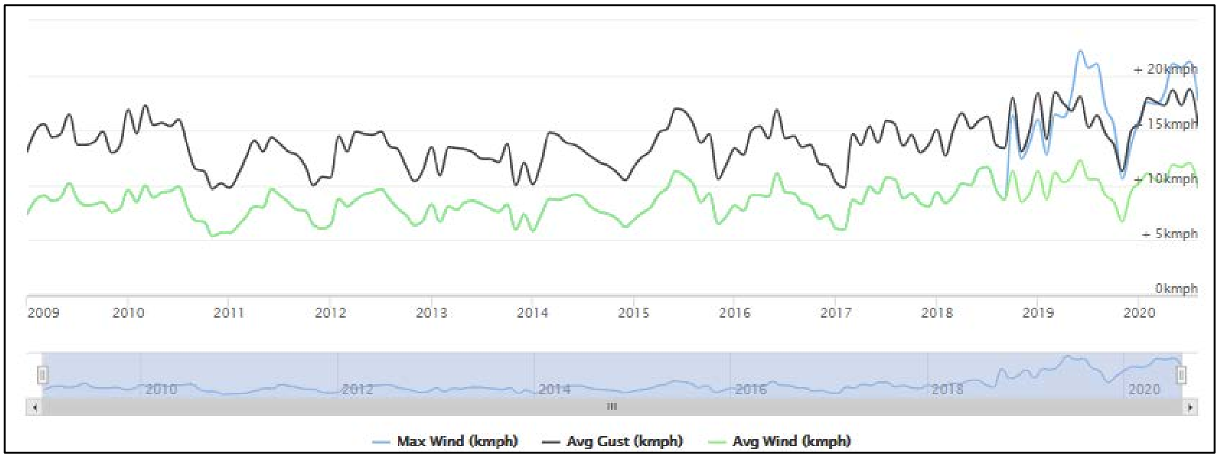
b)


1020mb
1010mb
1000mb
990mb
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
Pressure (mb)

c)


200mm
150mm
100mm
50mm
0mm
2009
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
Rain (mm)
Days

d)



e)

Figure 8. Variation of temperature (a), cloud and humidity (b), pressure (c), rainfall amount and rain days (d) and wind (e) in Erbil City (Erbil Monthly Climate Averages, https://www.worldweatheronline.com/erbil-weather-averages/arbil/iq.aspx)

4. Conclusions

Till 28 July 2020, 16,301,736 confirmed cases of COVID-19 were recognized by WHO, including 650,069 deaths. COVID-19 PL affected our life, movement, political, sports, business, industries, economy, oil, social relationship, journey, tourist, working hours, education etc. and it changed/inversed many things. It affected the natural environment as well. Results showed that COVID-19 PL increased a number of parameters, such as domestic and hospital wastes, noise at home, aquatic life and water quality, domestic WW amount, green areas, animal and birds movement, natural energy, rodents etc.; whereas, it decreased numerous factors, for example commercial/industrial solid waste, traffic and outdoor noises, air pollution and particles, water contamination, WW production, cutting trees and hunting, fuel extraction and mining, artificial light and radiation, fuel combustion, tourist and picnic etc.

Alternatively, some parameters such as black water, natural radiation, and ordinary lighting remained as before COVID-19 PL. Positive, nil, and negative influences of the factors on the environment caused by COVID-19 PL were 81.43 %, 5.71 %, and 12.86 %, respectively. Positive effects of the COVID-19 PL on the environment were superior than the negative impacts and lockdowns and can be regarded as a breathing period of the natural environment. In 2020, pressure, rainfall, and wind varied in Erbil City and they had the impact on microorganisms. Presently, forecasting of season influence on spreading COVID-19 is not easy.

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Potencijalni uticaj zabrane kretanja tokom COVID-19 pandemije na parametre životne sredine

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INFORMACIJE O RADU

Primljen 11 avgust 2020
Prihvaćen 15 oktobar 2020

Originalan rad

Ključne reči:
Kvalitet vazduha
COVID-19
Životna sredina
Grad Erbil
Zabrana kretanja
Zagađenje

I Z V O D

COVID-19 je imao uticaj na svakodnevni život, ljudske aktivnosti, različite sektore i životnu sredinu. Shodno tome, cilj ovog istraživanja je bio ispitivanje uticaja zabrane kretanja tokom pandemije COVID-19 virusa na parametre životne sredine. Ispitivani su sledeći parametri životne sredine: čvrst otpad, buka, vazduh, voda, otpadna voda, zemljane i zelene površine, prirodno okruženje i resursi, svetlosno zagađenje, zagađenje radijacijom, energija i drugi. Glavni problemi zaštite životne sredine su podeljeni na sedamdeset podparametara. Rezultati su pokazali da je pandemija COVID-19 virusa uticala na povećanje brojnih parametara, kao što su kućni i bolnički otpad, buka u kući, vodeni život i kvalitet vode, količina otpadnih voda u domaćinstvu, zelene površine, kretanje životinja i ptica, prirodna energija, glodari i drugi. S druge strane, pandemija je uticala na smanjenje nekoliko drugih faktora, kao što su komeracijalni/industrijski čvrsti otpad, buka u saobraćaju i na otvorenom, zagađenje vazduha i prisustvo čestica, zagađenje vode, zagađenje otpadnim vodama, seča drveća i lov, sagorevanje goriva i rudarstvo, turisti i drugi. Pored toga, neki parametri, poput crne vode, prirodne radijacije i normalnog osvetljenja, ostali su isti kao i pre pandemije COVID-19. Pozitivni, nulti i negativni uticaj parametara na životnu sredinu su iznosili 81,43 %. 5,71 % i 12,86 %. Pozitivni uticaj pandemije na životnu sredinu je bio veći od negativnog uticaja, a zabrana kretanja može da se posmatra kao period disanja prirodnog okruženja. Trenutno je teško predvideti sezonski uticaj na širenje COVID-19 virusa.

Recycling and Sustainable Development 13 (2020) 63-71

www.rsd.tfbor.bg.ac.rs

Recycling and Sustainable Development

RSD

Online ISSN 2560-3132
Print ISSN 1820-7480

Ecological dimension of PESTEL analysis in small enterprises in the Republic of Serbia

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ARTICLE INFO

Received 16 July 2020
Accepted 28 October 2020

Review article

Keywords:
PESTEL analysis
Business environment
Ecology
Ecological factors
Sustainable business

ABSTRACT

Strategic decision-making seeks to analyze the external environment in which business organizations operate. External environmental factors are analyzed and monitored using PESTEL analysis. PESTEL analysis provides insight into organization's business opportunities and threats, in order to rapidly adapt to new market conditions. The objective of this paper is to present the use and the importance of PESTEL model for the analysis of ecological factors in business environment. The most common challenges of environmental analysis, and examples of positive practice from the Republic of Serbia, are presented in this paper. The paper represents a professional contribution to the business community, specifically, it helps organizations to improve their ecological performances through more efficient use of opportunities and elimination of threats arising from ecological factors.

1. Introduction

Strategic decision-making in organizations seeks numerous information from internal and external environment. This involves conducting a strategic analysis, with emphasis on external environment assessment, based on assumption that organizations cannot adapt in short term to operating environment. Therefore, managerial task is to estimate the opportunities and threats from external environment, to make the best possible adjustments, taking into account organizational strengths and weaknesses. In order to succeed, managers should realize environmental structure with the help of various strategic tools, and one of the most important is PESTEL analysis.

PESTEL (political, economic, social, technological, environmental, and legal) analysis is an important tool used for market and environmental analysis and for supporting strategic decision-making (Narayanan and Fahey, 2001). By analyzing the factors of macro environment, organizations can focus their efforts on avoiding threats and embracing opportunities. The PEST concept was originally developed by Aguilar (1967) and included four general environmental factors (political, economic, social, and technological) (Jarzabkowski et al., 2009). Subsequently, new dimensions of general environment, such as legal and ecological, were added to understand their importance for the organizations' performances. The systematization of general environment according to PESTEL model has been accepted by most theorists, and the model is often used in practice. The aim of PESTEL analysis is to evaluate the influence of general environmental factors, taking into account their interdependence. Specifically, development of one factor influences the development of others, and this interdependence is emphasized by many authors (Collins, 2012; Yuksel, 2012; Ho, 2014).

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doi: 10.5937/ror2001063M

Academic research related to PESTEL analysis can be devided in three main segments: research related to the nature of PESTEL analysis; research on the relationship between PESTEL analysis and strategic management; research related to the application of PESTEL analysis in practice (Ho, 2014). Studies of the nature of PESTEL analysis are related to: categorization of general environmental factors into major groups (Clulow, 2005); inter-connection of different general environmental factors (Collins, 2012; Yuksel, 2012; Ho, 2014); relationship between organization and general environment (Fleisher and Benoussan, 2003); etc. Research analyzing the link between PESTEL analysis and strategic management investigates: key drivers (Darkow, 2014); the link between PESTEL and SWOT analysis (Shabanova et al., 2015); and the importance of PESTEL analysis for strategy design (numerous theorists). Research related to the application of PESTEL analysis in practice analyzes: data collected from managers of different hierarchical levels; data collected from employees engaged in strategic planning; and secondary data required for PESTEL analysis.

In contemporary world, ecology became a particularly important external factor, accompanied by sustainable development. It significantly emphasizes the increasing social responsibility of business organizations in terms of sustainable development, preservation, and protection of the environment. Ecological dimension of external environment certainly makes one of the key features of today's civilization. Ecological factors can be identified at different levels: globally (the ozone hole and global warming); regionally (acid rain, groundwater pollution, oil spills); and locally (water pollution, air pollution, problems with waste disposal). From the aspect of PESTEL analysis, ecological factors may include weather conditions, climatic conditions, environmental laws, regulations and acts, climate change, environmental requirements of NGOs, natural disasters, air and water pollution, recycling standards, public opinion regarding green production, and renewable energy support (Business to you, 2016).

The ecological goals of business organization are focused on products/services without harmful consequences, i.e. on improving the quality of environment by eliminating ecologically risky products/services or their individual elements. In addition, ecological goals include eco-packaging, emphasizing ecological arguments in advertising, informing, and educating consumers about the ecological dimension of products and services. These goals include systematic waste management, systematic measurement of cost-effectiveness of materials and energy with the aim of rationalizing consumption, the use of economical materials, as well as energy resources with minor environmental consequences. The ecological goals include introduction and application of production technologies for environmental protection, but also actions for optimizing logistics processes in ecological sense (reduction of transport and fuel consumption).

Small enterprises need to adopt appropriate management tools for analyzing business in the context of economic and ecologic factors coexistence, and improve ecological efficiency (Passetti and Tenucci, 2016). Ecological efficiency can be improved in several ways (WBCSD, 2006): reengineering process (reduction of resource consumption and pollution, cost savings); byproducts revaluation (exchange of byproducts establishes synergetic links with other organizations, whereby the waste of one organization becomes a raw material for another); product redesign (the product should be simpler, with as few different materials as possible); and changing market access (finding new ways to meet consumer needs).

The precondition for sustainable development of an organization is the acceptance of ecology as a “specific stakeholder”, the harmonization of business and ecological factors, and the achievement of ecological performances in business. The basis of this assumption is the positive impact of ecological factors on economic performances. Linking economic performances and ecological factors results in innovating traditional business performance indicators, in terms of defining new planned performance, primarily ecological efficiency. Ecological efficiency encourages business organizations to focus on opportunities arising from ecological factors, which will at the same time contribute to positive financial performance.

The objective of this paper is to present the theoretical and methodological framework of the PESTEL model; its application in external environment analysis; the need and importance of ecological factor analysis; along with emphasis on the most common challenges faced by small enterprises in the Republic of Serbia, when analyzing ecological factors in PESTEL model. The paper will deal with positive examples of small enterprises from the Republic of Serbia, in terms of efficient use of opportunities and elimination of threats arising from the influence of ecological factors. The aim of this paper is to point out the importance of ecological factors’ analysis in PESTEL model, to emphasize the importance of applying ecological dimension in strategic decision making, as well as to emphasize the need and importance of analyzing ecological factors, with the aim of achieving high competitive performances.

2. Materials and Methods

The realization of research goals was carried out by applying several research methods, and the following were predominantly applied: bibliographic-speculative method - in the first phase of research for the analysis of scientific-theoretical concepts of PESTEL model and ecological factors; and analytical-synthetic method - in the process of classification and interpretation of research results. Empirical material in research, that is the positive examples of small enterprises from the Republic of Serbia, was collected using the following research technique: semi-structured interviews that were applied in the case study.

The research encompassed primary data sources, as well as secondary ones. The collection of primary and secondary data involved the application of certain research methods and techniques. Primary sources were collected through semi-structured interviews, conducted in selected small enterprises, which based their business on ecological sustainability. Secondary sources were from archival data. A detailed investigation was conducted to detect small enterprises which included ecological dimension in their strategic decision making, and were able to spread their experiences through the semi-structured interviews. Targeted sampling method was used to select research participants. The criteria for participation were that each small enterprise had to have experience in ecological sustainability for at least three years before the research was conducted.

After the detailed investigation, the research covered three small enterprises operating in the Republic of Serbia, and they were the subject of a case study: Hotel Ramonda on the Rtanj mountain (Ramonda Rtanj doo Boljevac), DRENOVAC doo Mirosaljci (purchase, processing, and sale of deep-frozen and dried lyophilized fruit), and FEPLO doo Čačak (factory that produces ecological waterproof panels).

3. Results

Business organizations are challenged with ecological factors within the PESTEL analysis. Insufficient depth in the analysis of these factors leads to insufficiently good strategic decisions that lead to missing market opportunities. The analysis of ecological factors in PESTEL model is one of the key elements of designing future business. In the following text, several quality analyses of ecological factors from the external environment using the PESTEL method, in selected small enterprises from the Republic of Serbia are presented.

Table 1.
Example of ecological factors analysis for Hotel Ramonda Rtanj

External environment factor	Factor description	Opportunity	Threat
(E) Ecological factor	Construction of hotel with ecology friendly materials (stone and wood). Use of carpets, curtains, bedding and towels made of natural materials. Furniture made of natural materials. Energy efficient appliances, smaller energy consumers (dishwashers, washing machines and dryers, water heaters, refrigerators).	YES - Increasing the number of guests who want to be in perfect harmony with nature during their stay at the hotel. YES - A better image of the hotel in public leads to an increase in number of guests and income of the hotel. YES - Lower costs as a result of using energy efficient devices.	YES - Introduction of stricter environmental standards that could increase the cost of services and require additional investments and additional costs
(E) Ecological factor	Possession of chargers for electric vehicles. Promotion of vehicles with environmentally friendly engines. Promoting the use of bicycles and walking.	YES - Increasing the number of guests, owners of electric cars. Revenue growth.	
(E) Ecological factor	Completely ecological way of purifying the water of the outdoor pool. No chemicals are used to purify water, but plants purify water. In the upper pool, where underwater and abovewater plants, moss, algae and water lilies predominate, the water is purified and poured into the lower part, which is used for swimming.	YES - Increasing hotel attendance, increasing economic profits. Increasing tourist interest, positive financial effect in the long run. YES - Cost reduction due to a more economically viable way of cleaning the pool. YES - Possibility to obtain incentive funds from funds that invest in environmental improvement.	

Hotel Ramonda on the Rtanj mountain (Ramonda Rtanj doo Boljevac) is an example of a completely ecological wellness-spa hotel (https://ramondahotel.com/). It is built of wood and stone, and wastewater decomposes naturally without the use of chemicals. That is why the swimming pool is specially designed. Hotel Ramonda has approached sustainable development and environmental protection by introducing new technologies, equipment, and programs, in accordance with the principles of ecological responsibility. The principles of ecological responsibility of this hotel include waste reduction, reuse and recycling, efficient use, conservation and management of energy, management of clean water and wastewater resources, waste care, and ecologically friendly transportation. Hotel Ramonda offers ecologically responsible accommodation that follows the practice of ecological living. This emphasizes the ecological orientation of the hotel, which makes a significant contribution to its business performances. PESTEL analysis of ecological factors for Hotel Ramonda on the Rtanj mountain is presented in Table 1.

DRENOVAC doo Mirosaljci is a family business organization that deals with the purchase, processing, and sale of deep-frozen and dried lyophilized fruit, in accordance with all legal regulations and high standards (http://www.drenovac.co.rs/). DRENOVAC doo, with its business, contributes to the environment development where natural resources are limited, in which water is less and less and where biodiversity is declining. In 2014, the IFS FOOD standard was implemented, which led to the achievement of the following goals: increased level of safety and quality of products, more clear and easy operations through procedures and work instructions, more detailed traceability of products, strengthened customer trust, and increased number of customers. Accredited certification bodies, with highly qualified assessors, participated in the assessment of standards. In its business, DRENOVAC doo is dedicated to numerous activities that contribute to the preservation of environment. PESTEL analysis of ecological factors for DRENOVAC doo Mirosaljci is presented in Table 2.

FEPLO doo Čačak is a factory that produces ecological waterproof panels (http://www.feplo.rs/). Waterproof ECO panels produced in the FEPLO factory are 100 % ecological product because no glues, additives, and formaldehydes are used in the production process. The production process is completely ecological, and waste tetrapack is used as a raw material, which has so far ended up in landfills, so the product is 100 % ecological.

Table 2.
Example of ecological factors analysis for Drenovac doo Mirosaljci

External environment factor	Factor description	Opportunity	Threat
(E) Ecological factor	Full compliance with applicable environmental legislation.	YES - Sales growth and aquisition of new markets that require high standards in the field of environmental protection	YES - Increase costs to meet all legal requirements
(E) Ecological factor	Raising the level of environmental awareness through constant information, training and education.	YES - Growing ecological awareness leads to attracting new clients who were previously insufficiently informed about the importance of preserving the environment and sustainable business	YES - Increase in training and education costs, increase in marketing costs
(E) Ecological factor	Rational use of resources and energy.	YES - Reduction of input costs	YES - Increasing the costs of introducing new technological solutions in production
(E) Ecological factor	Waste management measures. Waste disposal in a safe way. Waste recycling.	YES - Facilitated access to financing waste recycling projects YES - Energy independence YES - Saving materials and energy through a complete production process	YES - Increase the cost of introducing new technologies for waste removal and recycling
(E) Ecological factor	Implementation of preventive measures with the aim of preventing the possibility of an ecological incident	YES - The possibility of an ecological incident is eliminated, the potential reduction of business revenues is eliminated.	YES - Costs of implementing preventive measures

Machines for the production of ECO panels were constructed by engineers employed in the factory, and the production is performed in a production plant that meets ecological standards. To make one “Feplo” panel, it is necessary to spend up to 20 kg of tetrapacks, so FEPLO uses it to preserve the environment. Every month, 250 tons of waste tetrapacks are installed in the production of ECO panels in the FEPLO factory. PESTEL analysis of ecological factors for FEPLO doo Čačak is presented in Table 3.

The analysis of external ecological factors, in presentation of organizations RAMONDA Rtanj doo Boljevac, DRENOVAC doo Mirosaljci, and FEPLO doo Čačak, was done in great detail with a very precise quantification of potential opportunities and threats. Each data in analysis was explained and argued on the basis of existing experience and research of future circumstances in external environment. Ecological factors of the external environment, that were important for future business, were identified. The opportunities and threats, arising from the impact of ecological factors on organizations’ core business, were presented in detail.

Table 3.
Example of ecological factors analysis for Feplo doo Čačak

External environment factor	Factor description	Opportunity	Threat
(E) Ecological factor	Production of 100 % organic product.	YES - Increasing the number of customers who want to use ecologically friendly construction materials in construction. YES - A good image of an ecological product leads to an increase in the number of customers and business revenues.	YES - Introduction of stricter ecological standards that could increase production costs.
(E) Ecological factor	Ecological process of making ECO panels, without the use of glues, additives and formaldehyde.	YES - Reduction of costs due to economically more profitable production process. YES - Possibility to obtain incentive funds from funds that invest in environmental improvement.	YES - Increase costs to meet all legal regulations for organic production.
(E) Ecological factor	Legislation that obliges to reduce the total amount of bio-waste that is disposed of.	YES - The use of bio-waste in the production process is economically viable. YES - Possibility to obtain incentive funds from funds that invest in environmental improvement. YES - Reducing the use of new raw materials and saving energy. Energy consumption in obtaining raw materials through recycling is significantly lower than the energy consumption that occurs during the processing of natural resources for obtaining raw materials.	YES - Increasing the costs of introducing new technological solutions in production, in order to monitor the requirements of new legal regulations.
(E) Ecological factor	Quality of ecological product (water resistance of ECO panels, good thermal isolation).	YES - Product quality enables high energy efficiency, which represents savings for the customer (advantage of the influx of new customers and increased business revenues).	YES - High costs for obtaining the necessary attestations and certificates (and for renewing them).

4. Discussion

The research was conducted with the aim of identifying the key ecological factors within the PESTEL analysis, i.e. factors that affected the application of ecologically sustainable business in small enterprises in the Republic of Serbia. The determination and selection of factors was realized by a detailed research and analysis of relevant literature, i.e. all published materials that were available, by conducting semi-structured interviews, and by performing the PESTEL analysis for three small enterprises that had already implemented ecologically sustainable business. All selected factors were analyzed from the aspect of opportunity or threat to the profitable business. Factors that represented opportunity for small enterprises and stimulated the development of their sustainable business were analyzed. Then, the factors that had a limiting effect on small enterprises and jeopardized their business were analyzed as well.

The results showed that the implementation of ecologically sustainable business was extremely important for small enterprises in the Republic of Serbia. Today, ecological sustainability is a key aspect of business, and implies an active application of all possible measures and activities in all areas of ecology protection. The implementation of ecologically sustainable business is a voluntary process for analysed small entreprises, except in the case of the existence of legislation that must be applied.

The research determined that ecologically sustainable business of small enterprises in the Republic of Serbia included several domains of ecological protection and preservation: pollution prevention, sustainable use of resources, climate change mitigation and adaptation, and protection and restoration of the natural environment (natural heritage, biodiversity). The research identified all the most important ecological factors within the PESTEL analysis, as well as measures and activities that should be applied in environmentally sustainable business in practice. The most important areas of measures and activities were related to energy management (energy efficiency and renewable energy sources), water management (reduction of consumption, purification, and reuse), and waste management (reduction, recycling and reuse, and safe management of hazardous waste), then ecological procurement, ecological building and equipping, organic food, reducing emissions and noise, protecting biodiversity and restoring ecosystems, ecological transport, creating partnerships and social responsibility, including cooperation and assistance to the local community, education and training of employees, customer participation, preparation of reports and environmental balances available to the public.

The research further determined that there were great potentials for positive implementation of ecological factors in small enterprises. Such implementation contributed to better competitiveness, image, reputation, loyalty of customers and employees, and thus to sales and achieving better economic and financial performances. The most important potentials of ecological dimension was based on: reducing negative impacts and preserving the environment, reducing operating costs and cost control efficiency, especially energy, greater customer satisfaction, greater brand reputation, and creating a better image and competitiveness.

Analyzed small enterprises that have taken the trend of sustainability seriously, use it profitably. Environmental management, which begins with the analysis of ecological factors in PESTEL model, can lead to significant benefits for business organization, as well as environmental benefits. Business organizations can reduce costs and increase productivity by reducing and managing resource use. Typical areas where cost savings are possible include the use of raw materials, waste, water, energy and transport, travel and packaging. By reducing environmental impacts, small enterprises can significantly reduce taxes or avoid associated costs. Responsible risk and liability management can also lead to a reduction in insurance costs. Small enterprises can achieve sales improvement as a result of strengthening their reputation among customers, informing them on relevant environmental issues in a clear and transparent manner. Good reporting increases customer confidence. Informing about the efforts made to preserve the environment can lead to an increased trust in products and services. That way small enterprises can achieve the preferred supplier status. Large corporations require ecological performances information from their suppliers and contractors, to meet the expectations of their shareholders. Ecological performances reporting can make small enterprises more attractive than competitors. Small enterprises can also become attractive to investors, who ask questions about business sustainability. Reporting on environmental issues provides a good indication that organization is taking measures to reduce risk and develop new opportunities. Business benefits can also be reflected through product and service innovations. Clear reporting on environmental management helps small enterprises to attract quality employees. Ecological reputation can be an important factor in choosing an employer. Environmental management and minimizing the organization's impact on the environment can improve relationships with legislators, and provide the business organization with an operating license, by providing the compliance with ecological legislation and other relevant laws and regulations.

5. Conclusion

Analysis of external environment in terms of ecological factors implies that the preservation of human health and the rest of living world should not be neglected as one of organizational goals in the process of strategic planning. Business organizations should have ecological awareness and their activities should contribute to the implementation of ecological programs and projects at the local level. The ecological awareness assumes that customers would choose the ecological product or service, in order to support the development of organization that offers such products/services. The ecological awareness also assumes that suppliers will base their deliveries on ecological products. The ecology field is regulated by laws and other legal rules that provide incentives and facilities for organizations with ecology awareness. PESTEL analysis of ecological factors should identify opportunities and threats in terms of ecological aspects of organization's business activities. This implies opportunities for investments in various ecological actions, programs, and initiatives in the future, provided that they are economically justified. Economic goals are the basis of every organization's business, but non-economic goals should not be neglected. This includes ecological goals, which are a prerequisite for sustainable development of business organization and society in general.

Focusing only on financial benefits often leads to irrational and reckless use of resources, and in general, to the neglect of business effects on the environment. There is a growing awareness of ecological environment protection, since it is without adequate alternative, despite all technological advances and achievements of the modern age. By integrating ecological analysis into the management decision-making process, business organization can reduce its costs, improve its competitive position, ensure regulatory compliance, and strengthen social responsibility, thereby building the organization's reputation. It is possible to be a competitive and profitable organization at the same time, and to operate with responsiblity towards society and ecology. The analysis of ecological factors, within PESTEL model, enables the business organization to integrate economic and ecological dimensions into everyday business, while contributing to the economic growth of organization and to the society progress.

Quality analysis of ecological factors, performed by PESTEL analysis, leads to successful use of business opportunities, while threats will be bridged by business solutions that can neutralize their negative impact. Spreading awareness of the importance of ecological protection is extremely important for small enterprises in the Republic of Serbia. This is supported by the fact that our country is on the path of reform and harmonization with European directives, on the path to EU membership. Opening the borders for foreign capital inflow for Serbian small enterprises, and the desire for recognition on the European and world market, impose a wider application of modern management techniques, and emphasize the importance of environmental management.

Ecologically sustainable business of small enterprises in the Republic of Serbia is a complex aspect of business, which requires a lot of activities and a systematic approach, bearing in mind that the effects on environment are great, as well as the complexity of the environmental process. This implies the establishment of environmental protection policy and strategy through ecologically sustainable business, then planning, implementation, control, and improvement activities. Successful ecologically sustainable business in small enterprises requires environmental management to be established, as well as reports and environmental balance sheets implemented and prepared. Efficient application of ecologically sustainable business in small enterprises in the Republic of Serbia requires the application of ISO 14001, an internationally recognized standard and tool for effective environmental management system, as well as EMS or EMAS, which establishes a complete system of sustainable management, planning, and environmental controls.

The research identified the most important areas, measures, and activities that should be applied within the ecologically sustainable business of small enterprises in the Republic of Serbia. It also identifies key areas that must be applied, and relate to energy, water and waste management, because of the greatest pollution in these areas. The most important and main specific principles in the application of operational ecologically sustainable business in small enterprises were identified and determined.

The research proves that the implementation of ecologically sustainability in small enterprises, in addition to environmental protection, achieves better financial performances, increases energy efficiency, and reduces operating costs, especially energy. Also, small enterprises benefit from a positive effect on creating greater competitiveness in the market, better image and reputation, help to achieve marketing benefits and promotion, which further has a positive effect on capacity utilization, i.e. better sales of products and services.

The research results clearly indicate that the application of ecologically sustainable business in small enterprises in the Republic of Serbia must be better and at a higher level, bearing in mind the importance of environmental protection, but also the popularity of this business in the world. The implementation of ecologically sustainable business should be implemented in every small enterprise in Serbia, because taking care of the protection and preservation of environment is the task of all of us. Given that small enterprises have the potential to increase their contribution to environmental process, minimizing negative environmental impacts can be realized in a number of ways. Many ecological activities do not require large financial investments, and can help a lot in protecting the environment. On the other hand, it brings benefits to small enterprises, which certainly refers to the cost reduction. This primarily includes programs such as: rational use of resources and energy, construction with ecology friendly materials, furniture made of natural materials, energy efficient appliances, promotion of vehicles with environmentally friendly engines, promoting the use of bicycles and walking, full compliance with ecological legislation, raising the level of environmental awareness through constant information, training and education, waste management measures, waste disposal in a safe way, and waste recycling. Therefore, every activity can contribute to the protection of environment, and according to its capabilities, every small enterprise should participate in it and give its maximum, because it is important that everyone contributes to environmental protection.

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Ekološka dimenzija PESTEL analize sprovedene u malim preduzećima u Republici Srbiji

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INFORMACIJE O RADU

Primljen 16 jul 2020
Prihvaćen 28 oktobar 2020

Pregledni rad

Ključne reči:
PESTEL analiza
Poslovno okruženje
Ekologija
Ekološki faktori
Održivo poslovanje

I Z V O D

Strateško donošenje odluka nastoji da analizira okruženje u kome posluju poslovne organizacije. Spoljašnji faktori životne sredine se analiziraju i prate pomoću PESTEL analize. PESTEL analiza pruža uvid u prilike i pretnje koje postoje za poslovanje preduzeća i na taj način omogućava brže prilagođavanje novim tržišnim uslovima. Cilj ovog rada je predstavljanje upotrebe i značaja PESTEL modela za analizu ekoloških faktora u poslovnom okruženju. Takođe su predstavljeni najčešći izazovi koji se javljaju prilikom analize životne sredine, kao i primeri dobre prakse u poslovanju preduzeća u Republici Srbiji. Ovaj rad pruža profesionalni doprinos poslovnoj zajednici, posebno organizacijama koje žele da poboljšaju svoje ekološke performanse efikasnijim korišćenjem prilika i uklanjanjem pretnji koje proizilaze iz ekoloških faktora.

Recycling and Sustainable Development 13 (2020) 73-86

www.rsd.tfbor.bg.ac.rs

Recycling and Sustainable Development

RSD

Online ISSN 2560-3132
Print ISSN 1820-7480

Analysis of Application of Aquaponic System as a Model of the Circular Economy - A Review

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ARTICLE INFO

Received 28 August 2020
Accepted 05 November 2020

Review article

Keywords:
Aquaponics
Republic of Serbia
Circular Eonomy

ABSTRACT

In a time of limited water resources, climate change, and significant reductions in fish and plant species, aquaponics systems can play an important role in the future of ecologically and socio-economically sustainable smart cities. The paper aims to investigate the available literature that deals with the topic and current situation related to aquaponics systems - their application, effects of work, perspectives, and shortcomings, as a model of the circular economy. The exploratory method includes a literature review and the analysis of interviews with the pioneers in aquaponics in the Republic of Serbia. The main criterion for reviewing the literature was to find successful examples of aquaponics in the world and the Republic of Serbia. The result of the research is that aquaponics systems, due to the circular way of production, can be included as the model of the circular economy. Among a growing number of aquaponics pioneers, BIGH Farm, ECF Farmsystems, Urban Farmers, Bioaqua Farm, Tilamur, and Water garden currently have a successful implementation of the circular economy. These firms produce food with no waste production, they minimize energy input, and have a positive environmental impact, which are the main aims of the circular economy.

1. Introduction

Nowadays one of the most significant challenges in environmental management across the world is ensuring that human activities conform to the principles of sustainable development (Xu et al., 2014). The circular economy is a system developed for minimizing the use of energy, natural resources, and waste generation (Tura et al., 2019). A circular economy for food consciously emulates natural systems of regeneration so that waste does not exist, but is instead feedstock for another cycle (Ellen MacArthur Foundation, 2019). In the past few years, the current problems in agriculture include the lack of arable land and limited water resources (Mateo-Sagasta et al., 2017; Palm et al., 2018), specifically in developing areas (Joyce, 2019). In a bid to address these problems, the idea of aquaponic system emerged. The circular economy introduces sustainable technological advancements to aquaculture (Hochman et al., 2018).

The idea of applying aquaponics can be useful in countries that have limited resources of agricultural production, a high rate of urbanization, and exponential population growth (Mchunu et al., 2018). Aquaponics has gained momentum due to its superior features compared to traditional production systems because aquaponics seems capable of maintaining ecosystems and strengthening capacity for adaptation to climate change, extreme weather, drought, flooding, and other disasters (Yildiz et al., 2019).

This means that aquaponics is gaining new and increasing attention as an important factor in achieving sustainable food production in the fight against insecurity

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doi: 10.5937/ror2001073A

and poverty, both in cities and in rural areas (FAO, 2015). Also, aquaponics is known as one of the "ten technologies which could change human lives" due to its potential to provide essential food for the growing urban population (Van Woensel et al., 2015).

Also, aquaponics is recognized as a solution for sustainable food production as it follows a biomimetic natural system and the circular economy principles by reusing water and nutrients (Goddek et al., 2015; Asciuto et al., 2019).

Aquaponics is a sustainable way of food production that combines aquaculture and hydroponics into a single circular system that mimics natural production systems (Radosavljević et al., 2014), while drastically reducing the negative impact on the environment (Blidariu et al., 2013; Danner et al., 2019).

The parameters and factors that need to be controlled when projecting and managing aquaponic systems are various and represent a major challenge when striving for the highest possible yields and quality (Reyes Yanes et al., 2020). Aquaponics is a complex system in which three different biological systems (fish, plants, and nitrifying bacteria) must be combined into one working system. For this system to function successfully, aquaponics combines several disciplines, such as aquaculture, microbiology, ecology, horticulture, agriculture, chemistry, and engineering (Yep and Zheng, 2019).

This recycling system, due to its significant advantages, has stimulated increasing academic research in recent years and aroused the interest of members of the public, as evidenced by the large number of papers related to this topic (Junge et al., 2019).

In numerous papers, researchers analyze the basic factors and parameters that must be taken into consideration in these systems - system design (Palm et al., 2018), the proper pairing of plant and fish species (Knaus and Palm, 2017), welfare and health of fish (Yildiz et al., 2017), the impact of media on crops cultivated (Oladimejia et al., 2020), water quality parameters (Sallenav, 2016), etc.

Because of the monitoring of the various factors, there is no complete and comprehensive critical analysis of aquaponics. The papers mainly focus on the analysis of one of these factors or on the possibility of improving existing systems and introducing new technologies (Suhl, 2020).

The number of papers and research in this field is limited for the territory of the Republic of Serbia (Radosavljević et al., 2014; Radosavljević et al., 2015; Blagojević et al., 2016).

Therefore, this paper aims to explain the basic principle of the aquaponic system, present its types, list the advantages and disadvantages, present examples from the world and the Republic of Serbia, and lay the foundations for further research of the aquaponic system in the Republic of Serbia.

2. Historical development of aquaponic system

Aquaponics is a hybrid name formed by combining the word aquaculture with hydroponics (Palm, et al., 2018). The earliest example of the use of aquaponics, according to some researchers, dates back to the time of the Maya and Aztecs, who grew plants on raised beams (rafts) on standing water surfaces (Jorge et al., 2011). The traditional name of this method of cultivation, among the Aztecs, comes from the word chinampas, which means an area surrounded by hedges or reeds, and today it is often translated as a floating garden (Figure 1), (https://fourstringfarm.com/2014/04/01/the-floating-gardens-of-aztecs). In addition to the Aztecs, the inhabitants of the area of southern China, Thailand, and Indonesia raised rice with the help of fish (carp, marsh eels, and river snails) and ducks (Figure 2), (McMurtry, 1988; Dai and Xuem, 2015). In China, rice production using aquaponic system dates back to the middle of the Han dynasty around 100 AD (Li, 1988).

Figure 1. Chinampas (https://fourstringfarm.com/2014/04/01/the-floating-gardens-of-aztecs)

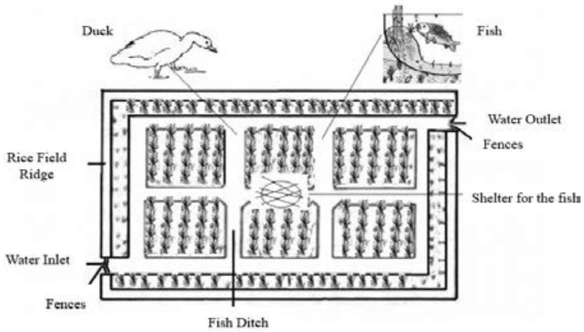

Figure 2. The symbiosis of rice, fish, and ducks (Dai and Xuem, 2015)

3. The operating principle of aquaponics

The circular process of the aquaponic system begins with feeding the fish in a fish tank. After a certain time, the fish excrete waste materials into the water, which are caused by the digestion of food. These products must be removed from the fish tank because their accumulation could be toxic for fish. The water with the fish's

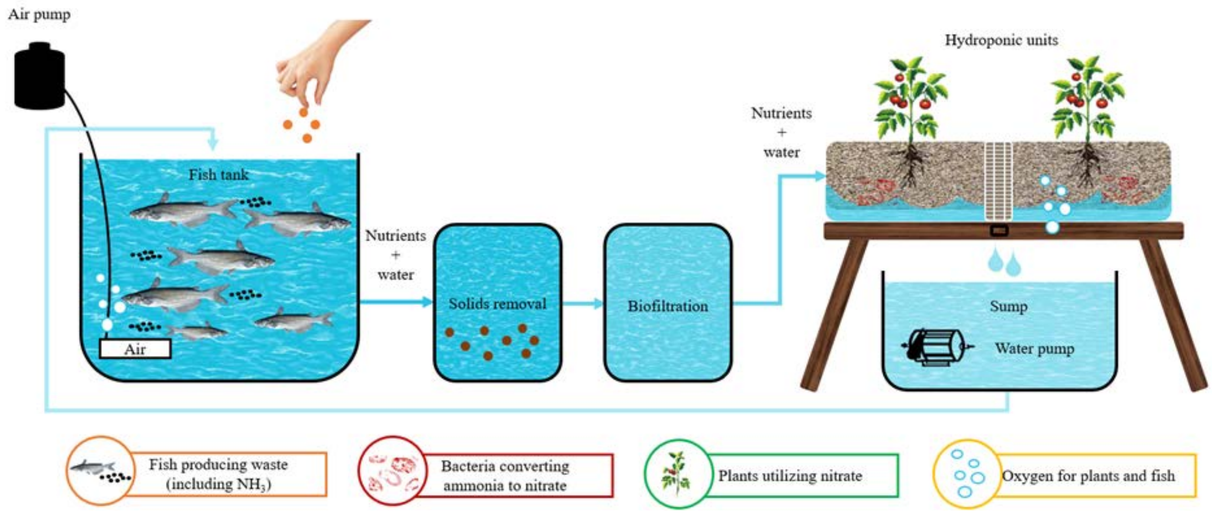

Figure 3. Schematic representation of the aquaponics system

metabolic waste goes to a mechanical filter that collects solid waste. The main task of this filter is to prevent the deposition of particles at the bottom due to the slow movement of water. After mechanical filtration, the water goes to the biofilter. The biofilter is designed to convert the ammonia (waste of fish) into nitrites and then nitrates; which can be consumed by plants. A biofilter is a permanent habitat for bacteria that convert fish waste into nutrients. From there, the water with dissolved nutrients is transported to the hydroponic unit in which the plants are placed, which absorb the nutrients from the water. Biofilters contain a medium such as gravel, sand, various plastic materials, etc. and their role is to ensure the "settlement" of bacteria (Turkmen and Guner, 2010).

At some aquaponic systems, there are no requirements for additional filter because the gravel, clay pellets, perlite, or some other internal substrates used, may act as a surface for bacterial colonization (Bernstein, 2011). The plants take all the nutrients from the water for their needs and act as filters. Namely, they purify the water and the clean water is pumped back to the beginning of the systems, to the fish tank. The process of nutrient removal prevents water from becoming toxic with harmful forms of nitrogen and allows plants, fish, and bacteria to live in symbiosis (Somerville et al., 2014). Thereby, all organisms work together to create a healthy environment for growth and development.

Products grown in aquaponics systems represent a closed cycle and thus avoid the generation of waste in agriculture (Proksch, 2019). The main component of these systems is water circulating between different elements (Krošelj, 2017). In a closed system, water is completely recycled. Plants grown in these systems consume only about 10 % of water (Bernstein, 2011). Figure 3 gives a schematic representation of the aquaponics system.

The crucial and most important part of the aquaponic system is bacteria, which serve as a bridge to connect fish waste with plant fertilizers. Namely, bacteria turn fish waste into fertilizer that plants use for growth. This two-step process involves bacteria of two genera (*Nistrosomonas and Nitrobacter*). Bacteria of the genus *Nitrosomonas*, in the first step, oxidizes ammonia, dissolved in water, turning it into nitrite. In the second step, bacteria of the genus *Nitrobacter* converts nitrites to nitrate that plants use, (Figure 4). Many authors in their studies investigate the influence of bacteria on faster plant development. Thus, Eck et al. (2019) gave a global overview of the diversity of bacterial communities, microbial properties, and potentials they can have in plant care. Fanga et al. (2017) studied the introduction of a colony of algae bacteria into aquaponics systems and the improvement of nitrogen efficiency utilization.

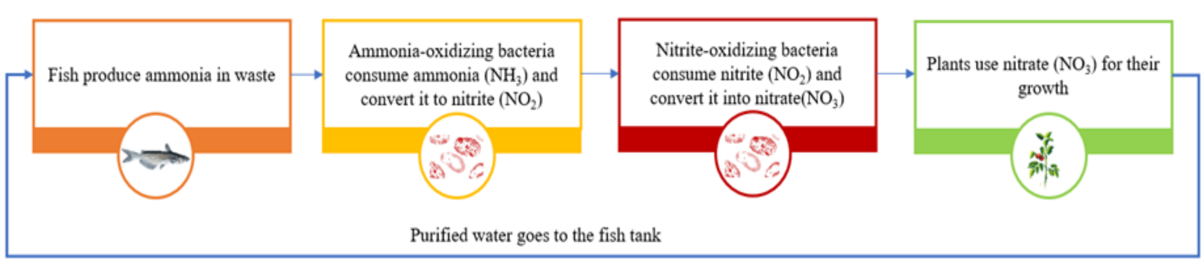

Figure 4. Nitrification process in the aquaponics system

When it comes to fish, the most commonly farmed species are carp, tilapia, ornamental fish, catfish, other aquatic animals, perch, pike, and others (Palm et al., 2019). In addition to these, in aquaponics systems, shrimp, crabs and the like can also be used (Love et al., 2015). Based on currently available research of aquaponics systems, it has not been determined whether the excretion of waste materials has a significant impact on the level of nutrients in the solution or on plant yield.

Further research in this field should help facilitate the selection of ingredients to be used in fish nutrition to improve fish growth and health and to increase yields of farmed plants (Robaina, et al., 2019).

To enable continuous and healthy plant growth in the aquaponics system, it is necessary to have ecological parameters of water and environment (Thorarinsdottir, 2015).

Water is also a very crucial element of the aquaponic system. Water quality and the chemical composition of water can affect each of the elements described above. Therefore, there is a need for continuous water quality and parameters monitoring. In Table 1 is given general water quality tolerances for nitrifying bacteria, fish (fish are grouped into hot and cold water fish), hydroponic plants, and ideal parameters for aquaponics as a compromise between all three organisms (Somerville et al., 2014).

Blagojević et al. (2016), analyzed the water quality in the area of Karlovac (Croatia), in the family home Ozimec, where a small aquaponic system of fish farming and food farming was made. The system was planted with river fish. Water quality was monitored during the breeding cycle. The authors monitored the values of the parameters: ammonia, nitrates, nitrites, and water, and established the optimal values of these parameters for this aquaponics system and determined how one parameter affects the others and vice versa. Tyson et al. (2008) analyzed how a change in pH value of water affects cucumber yield. It means that parameters and factors require a change, depending on the type of plants that will be grown. E.g., leafy vegetables are the most common type of plant grown in these systems because they grow well in concentrated water with nitrogen, have a short growth period, do not require a large number of nutrients and are generally in great demand in the world (Bailey and Ferrarezi, 2017). Commercial producers most often grow the following plant species: basil (Ferrarezi and Bailey, 2019), various types of lettuce, tomato, kale, paprika, and cucumber.

With additional care in these systems, eggplant and root plants, such as carrots (Bosma, 2017), onions, beets, and radishes (Somerville et al., 2014), can also be grown, as well as kale, thyme, barley, various types of flowers, etc. (Buzby et al., 2016).

Each element of the aquaponics system (plants, fish, and water) can be considered individually, also for each of them problems that may occur during the operation of the aquaponics system can be defined. Table 2 presents the problems that can occur with individual elements during the production process in an aquaponics system.

Table 1
General water quality tolerances for nitrifying bacteria, fish, hydroponic plants and ideal parameters for aquaponics as a compromise between all three organisms (Somerville et al., 2014)

Organism type	Temp	pH	Ammonia	Nitrite	Nitrate	Dissolved O_2
	[℃]	[mg/L]	[mg/L]	[mg/L]	[mg/L]	[mg/L]
Bacteria	14 - 34	6 - 8.5	< 3	< 1	-	4 - 8
Warm water fish	22 - 32	6 - 8.5	< 3	< 1	< 400	4 - 6
Cold water fish	10 - 18	6 - 8.5	< 1	< 0.1	< 400	6 - 8
Plants	16 - 30	5.5 - 7.5	< 30	< 1	-	> 3
Ideal parameters	18 - 30	6 - 7	< 1	< 1	5 - 150	> 5

Table 2
Problems with individual elements in the aquaponics system

Plant problems	Fish problems	Water problems
- Deficiencies and toxicity of some nutrients (Rakocy et al., 2006), - Plants are not growing and/or leaves are changing color (Somerville et al., 2014), - Nitrate levels are high yet plants leaves are yellowing (Somerville et al., 2014),	- Fish are piping at the water surface (Somerville et al., 2014) - Fish are not eating (Somerville et al., 2014), - Accumulation of fish in the system, consumption of space and food (Tyson et al., 2008), - The water temperature is too high or too low (Somerville et al., 2014),	- Temperature (Somerville et al., 2014), - pH (Somerville et al., 2014), - Nitrate or nitrite level (Somerville et al., 2014), - Carbonate hardness (Somerville et al., 2014), - Algae (Somerville et al., 2014), - Low dissolved oxygen (Somerville et al., 2014),

4. Basic cultivation techniques in aquaponics systems

Hitherto, in practice, three cultivation techniques in aquaponics systems are used: Nutrient film technique (Castillo-Castellanos et al., 2016), Media bed technique (Kamauddin et al., 2019), and Deep water culture technique (Somerville et al., 2014). The nutrient film technique (Figure 5) is a hydroponic method that uses horizontal pipes (Somerville et al., 2014). Through each pipe, there is a shallow stream of nutrient - rich aquaponic water. This water provides the necessary amounts of nutrients for plant growth. It is necessary to install a mechanical filter at the beginning, to avoid the accumulation of solids at the root of the plant. The water flows by gravity from the fish tank, through the mechanical filter and flows into the combination biofilter/sump. Through a “Y” connector and valves from the biofilter/sump, the water is pumped in two directions. Water can be pumped directly back to the fish tank, or it can be pumped into a manifold that distributes the water equally through the pipes. Again by gravity, the water flows down through the grow pipes.

The water outlet is at the end of the pipes. From the grow pipes water is returned to biofilter/sump, where again it is pumped either into the fish tank or grow pipes, thus ending the cycle (Somerville et al., 2014).

Plastic pipes are usually used as a material for these systems. Pipes are with a flat bottom and are angled by 1 %, to enable the gravity fall of water. Pipes dimensions are defined, and, usually, they should not exceed 10 m to avoid the loss of dissolved oxygen. Each pipe has holes, and the dimension of the hole matches the size of the available net cups. The plants are planted into the net cups, which are placed into the holes. These systems are suitable for growing lettuce and herbs (Maucieri et al., 2019), as well as for growing crops on concrete floors or roofs.

The advantage of these systems is the recirculation of nutrients and the absence of substrates. Thereby, there is a great potential for system automation while reducing operating costs and managing the optimal density of plants during its cycle. On the other hand, the lack of substrate and low water level in these systems creates the possibility of failure of the pump, or pipe clogging (Thorarinsdottir, 2015).

Media-filled bed units are the most popular design for small-scale aquaponics (Mullins et al., 2015). Figure 6 shows the main components of an aquaponic system using media beds, including the fish tank, the media beds, the sump tank, and the water pump. The media bed system is filled with suitable growing media such as expanded clay pebbles, lava rock/pumice stones or gravel. The water from the fish tank is pumped over to the media filled beds, and the plants grow in the clay or rock media. Many materials can be used as a medium in a media-bed system.

The media must be organic and have an adequate surface area to allow bacteria to grow and water to flow to the plants' roots. The first function of the medium is to help the root of the plant to more easily take nutrients from the water, and the second function is to use it as a filter, both mechanically (particle removal) and as biological (bacterial colonization). Gravel, spheres, coconut trust, vermiculite, etc. are most often used as a medium. There are three growing zones, based on the medium, within the bed: surface or dry zone, root zone, and zone of solid particle collection and mineralization (https://gogreenaquaponics.com).

This aquaponics system design has a relatively low starting price and can be expanded relatively easily with certain limitations. However, although they are classified as the simplest systems of growing crops, these systems also have certain weaknesses, e.g., water evaporation is higher in these systems (Thorarinsdottir, 2015).

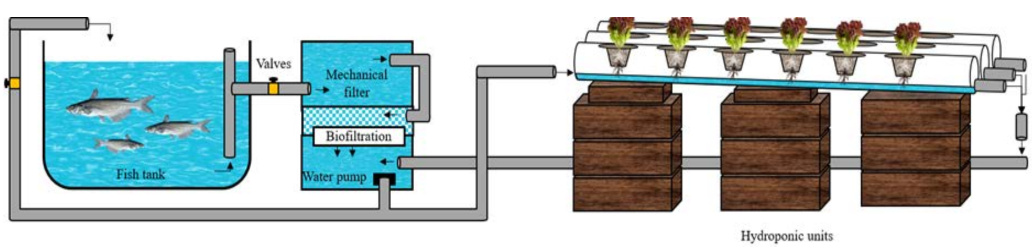

Figure 5. Nutrient film technique

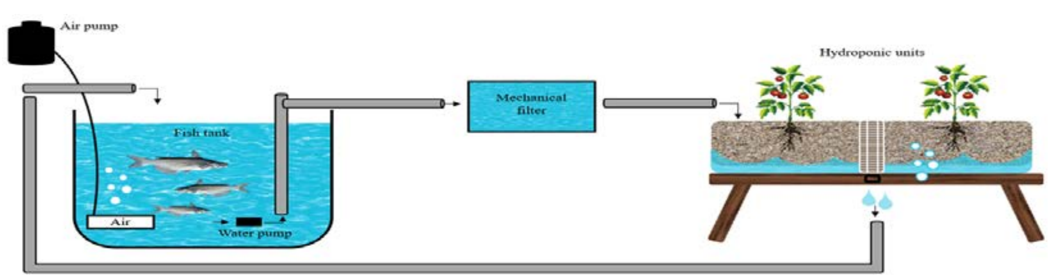

Figure 6. Media bed technique

Deep water culture techniques (Figure 7) are used for small and large aquaponic systems (Thorarinsdottir, 2015). The idea for this type of production is taken from Aztecs, who grew their crops in the same way. Deep water culture techniques are based on growing plants on floating or hanging supports (raft, plates, or boards) in troughs or containers filled with water with nutrient solution at a depth of 10 - 30 cm. Water containers are equipped with various floating material that serves to keep the plants above water, while the root of the plant itself is constantly submerged in water (Maucieri et al., 2019). The water flow dynamics in deep water culture techniques are almost identical to those through a nutrient film method.

The main difference between the nutrient film method and the deep water culture techniques is that in these systems, water is not drained from the container, namely, a large amount of water is constantly present, allowing the plants to use significant amounts of nutrients (Somerville et al., 2014).

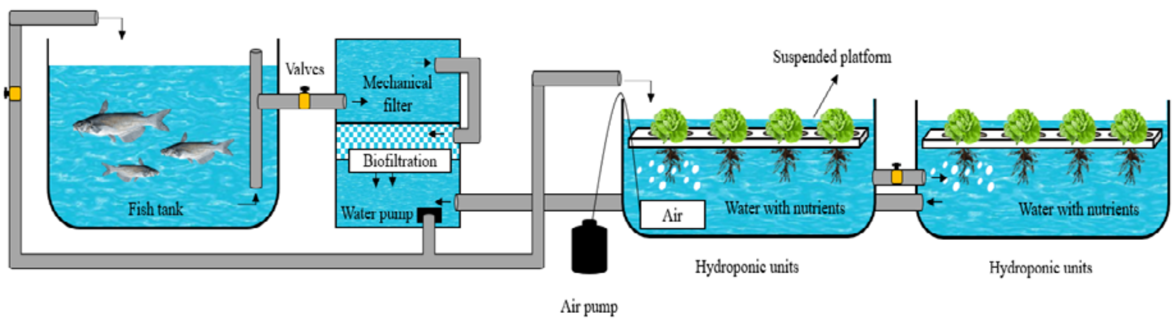

Figure 7. Deep water culture technique

Table 3
Strength and weaknesses of aquaponic systems

Strength	Weaknesses
– Organic food production (Acquacoltura Italia srl, 2016), – The possibility of applying cultivation in locations with infertile soil or without soil (Benko and Fabek, 2011), – Two agricultural products (fish and vegetables) are produced from one nitrogen source (fish food) (Somerville et al., 2014), – A wide range of cultivated aquatic animal and plant species (Acquacoltura Italia srl, 2016), – Higher yields and qualitative production (Somerville et al., 2014) , – Does not use fertilizers or chemical pesticides (Somerville et al., 2014), – There are no daily tasks, like as harvesting and planting are labor-saving (Somerville et al., 2014), – Rational water consumption (Somerville et al., 2014), – A small amount of waste is generated (Somerville et al., 2014), – Higher control on production leading to lower losses (Somerville et al., 2014), – Can be built in many ways according to the materials available (Somerville et al., 2014), – An integral part of sustainable cities (providing a better climate in institutions, landscaping, etc.) (Rizal et al., 2018), – Can be integrated into the local water cycle (use of rainwater, instead of freshwater) (Rizal et al., 2018), – Education (as an educational tool in schools) (Rizal et al., 2018), – Possibility of application of culture cultivation in space (mars) (Acquacoltura Italia srl, 2016), – Save time (hoeing, watering ...) (Rakocy et al., 2006), – Building materials and databases are widely available (Somerville et al., 2014),	– Expensive initial startup costs compared with soil production or hydroponics (Somerville et al., 2014), – Knowledge of fish, bacteria, and plant production is needed for each farmer to be successful (Somerville et al., 2014), – Fish and plant requirements do not always match perfectly (Somerville et al., 2014), – Limited types of the crop (El-Essawy et al., 2019), – Required techniques and knowledge to manage the use of equipment (Munoz, 2010), – Energy demanding (Somerville et al., 2014), – Requires reliable access to electricity, fish fingerlings, and plant the seed, especially for large aquaponics systems (El-Essawy et al., 2019),

The disadvantage of these systems is the provision of a stable and clean system due to the deposition of organic matter at the bottom of water containers, as sludge can accumulate on plant roots, so the plant can use a small amount of oxygen and nutrients from water (Palm et al., 2019). This type of hydroponic system is suitable for perennial crops, such as tomatoes, peppers, eggplant, cucumbers, zucchini, beans, etc. (FAO, 2013). In addition to the above, new methods are being developed for growing crops, such as aeroponic. The aeroponic technique is mainly aimed at smaller horticultural species and has not yet been widely used due to the high investment and management costs. Plants are supported by plastic panels or by polystyrene, arranged horizontally, or on inclined tops of growing boxes. These panels are supported by a structure made with inert materials, to form closed boxes where the suspended root system can develop. The nutrient solution is directly sprayed on the roots. The spray duration is from 30 to 60 s, whilst the frequency varies depending on the cultivation period, the growth stage of the plants, the species, and the time of day (Maucieri et al., 2019).

Although aquaponics offers many advantages compared to traditional agricultural systems, there are certain disadvantages, i.e. limitations in the spread of aquaponic cultivation techniques. Table 3 presents the strength and weaknesses of aquaponic systems.

There are currently no specific regulations or policies for the application of the aquaponics system in the European Union or most of its Member States. One of the reasons is that this area belongs to different ministries that are responsible for them (industrial aquaculture, wastewater recycling, hydroponics, and urban aquaculture), where producers are subject to potentially various and conflicting regulations (Reinhardt et al., 2019).

5. Application of aquaponics systems in the world

5.1. The first aquaponics farm - Brussels, Belgium

The Brussels star-up BIGH (Building Integrated Green Houses), which has the task of building a network of sustainable Aquaponic urban farms in larger cities, has opened its first large farm in Brussels. The farm combines a 2,000 m^2 greenhouse and a 2,000 m^2 outdoor garden, located on the roof of the Foodmet market hall (Figure 8). The company has developed an aquaponics system where fish, fruits, vegetables, and herbs are grown in a closed and zero waste loop. This farm applies the latest sustainable technology and takes into account the energy losses in buildings, as well as the use and recycling of rainwater and the use of renewable solar energy (https://bigh.farm). In one year, the fish farm can produce 35 tons of high quality striped bass fish per year.

The greenhouse produces herbs, tomatoes, and microgreens. The first 700 m^2 of the outdoor garden has been cultivated since 2016, and its total area of 2,000 m^2 will be developed over time.

Production capacity depends on varieties, sizes, and temperature (https://bigh.farm).

Figure 8. Greenhouse and outdoor garden (https://bigh.farm; https://agenda.brussels/en/468756/tour-of-ferme-abattoir-the-biggest-urban-farm-in-europe; https://www.construction21.org/infrastructure/be/abattoir-bigh-farm.html)

5.2. Efficient City Farm (ECF) - Germany

ECF was founded as a private limited company in 2012. Its goal was to implement aquaponic systems in farming, especially within the urban environment with close market proximity. They started building its prototype aquaponics farm in Berlin in 2014, and the farm started with production in 2015. ECF is a food producer that serves 2 distinct markets - supermarkets and HoReCa (Hotels, Restaurants, and Catering). ECF's aquaponics farm brings together in one urban location, fish farming (invites), and plant cultivation (in a greenhouse). It concentrates on one species in the respective domains - tilapia and basil (Figure 9), (Figeac, 2019).

Figure 9. ECF's aquaponics farm (http://www.ecf-farmsystems.com/en)

On this farm, they use two circles in aquaponics: the aquaculture circle for fish and the hydroponic circle for plants. This offers three decisive advantages - two different pH values can be set, optimized for the respective circuit. In hydroponics, minerals that are important for the plants can be added as substitution fertilizers without harming the fish. Each circuit can be switched off independently of the other for cleaning and maintenance purposes, which minimizes the risk of production. The water is used twice, and the fish excrements are used as fertilizer for the plants (http://www.ecf-farmsystems.com/en).

5.3. Urban Farmers - The Hague, Netherlands

The roof and sixth floor of De Schilde, a former Philips factory in The Hague, have given way to the large European aquaponics farm Urban Farmers, (Figure 10). The construction consists of a 1,200 m^2 greenhouse on the rooftop and 900 m^2 of space for fish cultivation on the floor below. Together, they form a perfect symbiotic system for fish and vegetable production within the city, in which the dirty water from the fish tanks is pumped into the planter beds to feed the plants, which in turn filter the water for the fish. Both floors also house irrigation systems, technical installations, and fish and vegetable processing rooms, (http://www.spaceandmatter.nl/urbanfarmers).

Figure 10. Aquaponic garden on the roof of a building in the Hague (http://www.spaceandmatter.nl/urbanfarmers)

5.4. Bioaqua farm - United Kingdom

Near Frome in Somerset, UK, there is a large aquaponic farm - Bioaqua farm (https://www.newfoodentrepreneurs.org.uk/index.php/training-support/61-bioaqua-farm), (Figure 11). The farm has a total area of 4,000 m^2, of which the aquaponics system covers an area of 1,700 m^2 and the orchard about 2,000 m^2. Investment costs were about € 111,000.

Figure 11. Bioaqua farm - UK (Bioaqua farm, 2016)

About 2 tons of trout and about 6 tons of various fruits and vegetables are produced annually (Bioaqua farm, 2016). The process uses about 95 % less water than is needed to grow plants by traditional methods (https://www.fresh-range.com/bristol/producer/bioaquafarm).

On this location vegetables, fruits, and fish are grown together. Also, a small part of the farm is used for experimenting with various ecological techniques to provide zero waste cultivation. The water used in the aquaponic garden is also used to irrigate the orchards. Besides, 4 rainwater collection pools contain about 2,000 oblong trout.

5.5 Tilamur - Spain

Tilamur is a Spanish company that was founded in 2012. The implemented technology is based on a special design conceived with the aim of quick and easy installation of components that enable the production of sustainable food for humans, (Figure 12). This model of the circular economy consists of a connected aquaponics system divided into three different areas. Due to its optimized design and low power consumption, this system can operate autonomously using photovoltaic and wind technology. The model is completely scalable, and the smallest design has a total greenhouse area of 400 m^2 in which about 2,000 kg of fish (tilapia), 3,320 kg of tomatoes, and up to 137 kg of algae (Spirulina) are produced annually. This amount of production can feed 100 people in one year, following the nutritional needs dictated by the FAO, (http://tilamur.com/es/nuestras-instalaciones/).

Figure 12. Aquaponics system in Tilamur (http://tilamur.com/es/nuestras-instalaciones/)

5.6. Water garden - Republic of Serbia

In the Republic of Serbia, aquaponics is still not widely represented. In the settlement of Stapar, in Sombor, in the Zapadnobački district, there is one of the few households that applies an innovative ecological method of production - aquaponics, (Figure 13).

This household is a pioneer in aquaponics farming, not only in Serbia but also in the region. On 600 m^2 of land, this household has 2 greenhouses that approximately occupy an area of about 200 m^2.

During one growing season (April-October) about 3-4 tons of tomatoes, about 500-900 kg of strawberries, and about 700 kg of carp are produced on an area of 70 m^2.

About 700 kg of carp is produced in three fish tanks (each 1 m^3) in 9 m^3 of water. The rest of the area goes to bio rectors, fish tanks, and passages (S. Radin, personal communication, 10.08.2020.). The process of production begins with feeding the fish, which introduces proteins into the system. At the end of the circular process, proteins are found in the food. From the fish tank, water is pumped into the hydroponic unit. In the hydroponic unit, water leaks through the growth medium, next to the plant roots, and after that, water is drained back into the fish tank.

Plants extract necessary nutrients for their further growth from the water and, at the same time, purify the water. From vegetable crops, in these greenhouses, tomatoes, strawberries, and Dutch lettuce are produced. The plant (tomatoes) has a rapid growth and can reach a height of about 4 m in one growing season, but due to the height of the greenhouse, the height of the plants is usually limited to 2-3 m (S. Radin, personal communication, 10.08.2020.). For this method of cultivation, there is no need for hoeing, watering, etc., so all attention is paid to the needs that plant requires.

The vertical system of aquaponic is implemented for strawberry production. About 1,000 strawberry seedlings were planted on only 3 m^2, and during the one growing season, it can produce about 500 - 900 kg of strawberries. In vertical systems, water comes to the seedlings from above, like artificial rain, provides a sufficient amount of oxygen and moisture for the plant. Dutch lettuce is also grown experimentally in the part of the greenhouse as the raft system in which plants float on styrofoam. About 15 kg of lettuce is produced within 15-20 days.

The growth and development of the plant are up to 6 times faster, and the yield is up to 3 times higher (Radin, 2020.). The whole system represents one ecological chain which results in much faster development of plants precisely because of that organic matter that is presented in the water. In such systems, the fish disease has not yet been investigated. Over 90 % of plant diseases are eliminated (because plant diseases are transmitted through the soil), and the remaining 10 % are treated preventively, with agents based on essential oils. Also, plants need to be protected from pests, insects, etc.

The material used to make this system is from the domestic market and is completely safe for food production. Different plumbing materials are most often used, while some of them have been remodeled and adapted to this method of cultivation. The average investment per 1 m^2 is about 130 €, without a solid base and greenhouse. About 3 million dinars were invested in this production, and the system consumes very small resources during production, such as the purchase of seedlings, juvenile fish, fish food, and electricity and water costs. On average, the sale of fish provides free crop production (S. Radin, personal communication, 10.08.2020.).

Premium final products are currently being produced and made - tomato juice, smoked fish steak, and strawberry jam.

Figure 13. Tomato production in small aquaponic farm in the Republic of Serbia (S. Radin, personal communication, 10.08.2020.)

6. Conclusions

Aquaponics is a modern, young, and sustainable new agricultural industry, which depends less on primary energy and material inputs, than conventional production systems. Also, aquaponic systems with their circular process fit perfectly into the principles of the circular economy and provide for a reduced impact of the agri-food sector in the environment. Thus, this technique involves the cultivation of vegetables without the use of land, and with a decrease in water consumption of up to 90 %. There are various types of aquaponic systems, and their advantages are enormous, in particular, because they contribute to the concept of the circular economy.

Even though the expansion of aquaponic, is significant, in practice, the use rates of aquaponics technologies are still low. Aquaponics businesses operate in a vague policy environment that falls under both aquaculture and agriculture. Thus, neither the EU nor the Republic of Serbia have the regulation for aquaponic.

Our literature review demonstrates that many countries and cities use aquaponic systems for the cultivation of different plant and fish species. The leading aquaponic firms include BIGH Farm in Anderlecht, ECF Farmsystems in Berlin, Urban Farmers in Hague, Bioaqua Farm in Blackford, United Kingdom, and Tilamur in Spain. They are among a growing number of aquaponic pioneers. In the Republic of Serbia, the pioneer is a small aquaponics farm in Stapar, in Sombor.

This small aquaponic farm on only 3 m^2, during the one growing season, produces about 500-900 kg of strawberries. The Republic of Serbia has the potential to develop more aquaponics systems and should use this potential to get closer to other countries that successfully use these systems.

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Analiza primene akvaponskog sistema kao modela cirkularne ekonomije – pregled

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INFORMACIJE O RADU

Primljen 28 avgust 2020
Prihvaćen 05 novembar 2020

Pregledni rad

Ključne reči:
Akvaponija
Republika Srbija
Cirkularna ekonomija

I Z V O D

U vremenu ograničenih vodenih resursa, klimatskih promena i značajnog smanjenja broja vrsta riba i biljaka, akvaponski sistemi mogu igrati važnu ulogu za budućnost ekološki i socio-ekonomski održivih pametnih gradova. Cilj ovog rad je da istraži dostupnu literaturu koja se bavi temom akvaponskih sistema i da ispita trenutnu situaciju u vezi sa ovim sitemima – njihovu primenu, rezultat rada, perspektivu i nedostatak ovog modela cirkularne ekonomije. Istraživačka metoda uključuje pregled literature i analizu intervjua sa pionirima u akvaponiji u Republici Srbiji. Glavni kriterijum prilikom pregleda literature je bio pronalaženje uspešnih primera akvaponije u svetu i Republici Srbiji. Rezultat istraživanja je pokazao da se akvaponski sistemi, zbog kružnog toka proizvodnje, mogu posmatrati kao model cirkularne ekonomije. Među sve većim brojem pionira u akvaponiji, kao primeri sa uspešnom primenom cirkularne ekonomije, nalaze se BIGH Farm, ECF Farm Systems, Urban Farmers, Bioaqua Farm, Tilamur i Vodena bašta. Ova preduzeća proizvode hranu bez stvaranja otpada, potrošnja energije je smanjena na minimum i imaju pozitivan uticaj na životnu sredinu, što predstavlja osnovne ciljeve kružne ekonomije.

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