Journal of Ecology and Toxicology

Review Article Open Access

A Probabilistic Rule for Ecology and Evolution: the Humpbacked Species Richness-Curve Aerobic conditions Digesters' Microbiology Ecology: The Primary Activated Sludge Process Producers

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Abstract

Wastes are treated via anaerobic digestion to lessen their potentially harmful effects on the environment. Complex organic chemicals are broken down into simple, chemically stable components, primarily methane and CO2, as a mutualistic activity of diverse anaerobic microbes. The collaboration of four distinct kinds of microorganisms, specifically fermentative, syntrophic, acetogenic, and methanogenic bacteria, allows for the conversion of complex organic molecules to CH4 and CO2. Different strategies are used by microbes to avoid unfavourable conditions in anaerobic digesters, such as competing for the same substrate between sulfate-reducing and methane-forming bacteria. Both the hydrogenotrophic and acetoclastic pathways can be used by methanobactin to produce methane. This review focuses on methanogenesis-related factors such as cellulosic microorganisms, cellulose structure, inoculum to substrate ratio, and source of inoculum. The use of molecular tools for tracking dynamic changes in microbial communities, such as denaturing gradient gel electrophoresis (DGGE), and fluorescent in situ hybridization (FISH), which examines taxonomy, interactions, and the dispersion of tropical groups, is also explored.

Interrelations involving species richness might be monomodal, monotonically growing, monotonically declining, bimodal, multimodal, U-shaped, or without any clear pattern at all. The most intriguing associations are the unimodal ones because they point to active, out-of-equilibrium community dynamics. They are controversial because of this. The literature on unimodal (humpbacked) species richness-relationships is reviewed in-depth in this article. Although less common than previously believed, unimodal patterns of species richness are frequently linked to disturbance, predation and herbivory, productivity, geographic heterogeneity, environmental gradients, temporality, and latitude. We investigate unimodal species richness-curves involving plants, invertebrates, vertebrates, plankton, and microorganisms in marine, lacustrine, and terrestrial habitats in order to better understand how these unimodal patterns vary depending on the creature and environment. Understanding contingent patterns and the many, interconnected mechanisms that produce them is one of the objectives of future research.

Keywords: Microorganisms; Herbivory; Hydrogenotrophic; Methanogenesis

Introduction

Methanogenesis is a series of intricate, reactive biochemical processes that take place in anaerobic environments. This procedure often entails the liquefaction and hydrolysis of intractable substances as well as the gasification of intermediates. An organic substance's partial or total mineralization and humification follow. The creation of biogas, a high energy fuel that may be utilised to produce environmentally friendly energy, is a benefit of the anaerobic digestion process. The main reason why scientists and businesses in the power sector have been interested in anaerobic digestion for about 140 years is this. Animal excrement, sewage sludge, various organic wastes from the food sector, or the organic portion of municipal garbage are just a few examples of organic wastes that can be digested using biotechnology to produce biogas [1]. Plants specifically cultivated for anaerobic digestion, like maize, are found in several countries. Currently, the production of biomass as a substrate for biogas plants is widespread throughout several European nations. The German government took action in 2011 to curtail even the production of maize grown in monoculture for energy purposes, which is the most extreme situation in Europe.

The production of biogas may be related to digestion in three ways. It is a technique for transforming the energy included in biomass into a usable fuel (biogas) that can be stored and delivered, first. As valuable liquid fertiliser and energy, it is also a technique for recycling organic wastes into stable soil additions. Thirdly, it is a form of waste treatment intended to lessen the dangers to the environment from those wastes [2].

A group of anaerobic bacteria work together to break down organic materials to produce biogas, a digester gas. Its composition, which is as follows, is dependent on the kind of raw material that is put through the digestion process as well as the manner used to carry out this process. Carbon dioxide CO2 (25–45%), methane CH4 (50–75%), hydrogen sulphide H2S (0–1%), hydrogen H2 (0–1%), carbon monoxide CO (0–2%), nitrogen N2 (0–2%), ammonia NH3 (0–1%), oxygen O2 (0–2%), and water H2O (2–7%) Are the other gases that are present in the atmosphere [3]. The biogas produced can be applied to a number of economic sectors, primarily in technical processes and for the following power engineering uses.

(1) Production of thermal energy in gas boilers and production of thermal and electrical energy in associated units (1 m3 of biogas produces 2.1 kWh of electrical energy and 2.9 kWh of heat); (2) production of electrical energy in spark-ignition or turbine engines.

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Received: 02-Jul-2022, Manuscript No: jety-22-71190, **Editor assigned:** 06-Jul-2022, PreQC No: jety-22-71190 (PQ), **Reviewed:** 20-Jul-2022, QC No: jety-22-71190, **Revised:** 22-Jul-2022, Manuscript No: jety-22-71190 (R), **Published:** 29-Jul-2022, DOI: 10.4172/jety.1000132

Citation: Joe J (2022) A Probabilistic Rule for Ecology and Evolution: the Humpbacked Species Richness-Curve Aerobic conditions Digesters' Microbiology Ecology: The Primary Activated Sludge Process Producers. J Ecol Toxicol, 6: 132.

(3) Use of the obtained gas as a fuel in motor vehicle engines.

(4) Use in a variety of technological processes, such as the production of methanol.

From 1 kilogramme of dry organic matter, the average efficiency of methanogenesis produces 0.24 m3 of methane. A m3 of biogas with a calorific value of 26 MJ can replace 0.77 m3 of natural gas with a calorific value of 33.5 MJ, 1.1 kg of hard coal with a calorific value of 23.4 MJ, or 2 kg of firewood with a calorific value of 13.3 MJ [4].

Ecologists have been looking for universal rules that apply to the entire natural world for many years. Examples of these efforts to identify overarching principles in ecology include the humpbacked, or unimodal, correlations between species richness and productivity and between species richness and disturbance. Following Hutchinson's well-known reflection on the vast number of animal species, several attempts were made. However, the form of the links between species richness and productivity and between species richness and disturbance has given rise to a great deal of debate, numerous models, and few generalisations. Ecologists had hoped for unimodal species richness curves to be the only pattern, but instead we observe a variety of patterns. Nevertheless, they are "contingent rules" because they depend on the organism and environment and happen frequently enough to be interesting.

Along with species abundances, one of an ecological community's defining traits is the number of species it contains (also known as its species richness). Others, particularly in severe conditions at high latitudes and altitudes, may only have tens of species. This is notably true in communities that are found in the tropics, where certain communities might have thousands of species. Community ecology's main challenge is figuring out why one community has more species than another [5]. The species richness-problem, however, is incredibly challenging due to the great intricacy of ecological relationships. In this article, we present a thorough assessment of the literature on one element of the species richness problem—humpbacked species richness relationships—including its history and recently developed new theories.

An ecological community's species richness and one of their traits, such as disturbance, predation severity, productivity, environmental gradients, time, or latitude, are functionally related through a connection known as a humpbacked species richness-curve. Unimodal, multimodal, monotonically rising, monotonically declining, and no relationship are among the minimum of five conceivable functional relationship categories. Although uncommon, U-shaped and J-shaped functions are also possible and most likely consist of a blend of monotonically increasing and decreasing functions [6]. Even though they are very distinct, Whittaker categorises U-shaped functions as unimodal. They are also not further discussed because there is no theoretical support for them in the literature on species richness.

Our review is not thorough and mostly concentrates on local species richness-functions, not regional or global ones or probability distributions. Therefore, we discuss local, elevational, and depth gradients but not global, latitudinal gradients though not among ecoregions as a whole, we talk about species richness within groups that make up ecoregions. The ecological communities we discuss share a majority of their species, which cannot be stated of communities in ecologically distinct ecoregions.

A species richness-observed curve's shape can be modulated by the processes that produce unimodal species richness-curves as well as the scale of investigation, whether it is fine or coarse. At one size, a unimodal curve might be seen, while at another, a monotonically growing curve might [7]. We talk about the ideas of grain, focus, and extent as well as alpha, beta, and gamma diversities.

Along with the scale issue, some taxonomic and functional categories of organisms are more likely to exhibit unimodal curves in specific settings. The unimodal species richness-curves of plants, invertebrates, vertebrates, plankton, and microorganisms in marine, lacustrine, and terrestrial ecosystems are covered.

Materials and Method

Stages of Anaerobic Degradation of Organic Wastes

Numerous bacterial species, including hydrolytic, acid-forming, acetogenic, and methanogenic bacteria, which create CO2 and CH4 as the major byproducts of the digesting process, are involved in the microbiology of anaerobic transformation of organic wastes.

The phasing of methane digestion is a distinctive feature. Each of them is responsible for the breakdown of a certain class of chemicals.

Hydrolysis

Fats are broken down into soluble monomers and dimers, or monosaccharides, amino acids, and fatty acids, during the hydrolysis of the polymerized, largely insoluble organic molecules, such carbohydrates and proteins. Extracellular hydrolases from the group (amylases, proteases, and lipases) produced by the right strains of hydrolytic bacteria are used in this stage of methanogenesis. It is believed that the hydrolysis of rarely decomposable polymers, such as cellulose and cellulo-cotton, inhibits the pace at which wastes are digested. Only 50% of organic molecules undergo biodegradation during the digestion of solid wastes [8]. Due to a lack of enzymes involved in their breakdown, the residual portion of the compounds persists in its initial state.

The creation of enzymes, their diffusion, and their adsorption on the waste particles subjected to the digesting process are all factors that affect the rate of hydrolysis. Other factors include particle size, pH, and the manufacturing of these enzymes. Bacteria from the category of related anaerobes belonging to genera like Streptococcus and Enterobacterium are responsible for hydrolysis.

Acidogenesis

The acidifying bacteria use this stage to transform water-soluble chemicals, including those left over after hydrolysis, into short-chain organic acids (formic, acetic, propionic, butyric, and pentanoic), alcohols (methanol, ethanol), aldehydes, carbon dioxide, and hydrogen. Proteins break down into amino acids and peptides, which can serve as a source of energy for anaerobic microbes. Due to the influence of different populations of bacteria, acidogenesis may have a bidirectional effect. Hydrogenation and dehydrogenation are two categories into which this process can be subdivided. Acetates, CO2, and H2 are the primary intermediaries in the transformation process; other acidogenesis products have a minor impact. Methanogens may directly employ the new products as substrates and energy sources. The bacterial response to a rise in hydrogen concentration in the solution is the accumulation of electrons by substances like lactate, ethanol, propionate, butyrate, and highly volatile fatty acids. Methanogenic bacteria cannot consume the new products directly; instead, they must be transformed by obligatory bacteria that produce hydrogen through a process known as acetogenesis. Ammonia and hydrogen sulphide, two acidogenesis byproducts that give this stage of the process an extremely awful smell, should also be mentioned by using oxygen that was unintentionally introduced into the process, the facultative anaerobes' acid phase bacteria promote the growth of the following genera' obligatory anaerobes: Pseudomonas, Bacillus, Clostridium, Micrococcus, or Flavobacterium.

Acetogenesis

Acetate bacteria, such as those from the genera Syntrophomonas and Syntrophobacter, convert the products of the acid phase into acetates and hydrogen, which can then be utilised by bacteria that produce methane. Methanobacterium suboxydans, a type of bacteria, is responsible for the breakdown of pentanoic acid into propionic acid, while Methanobacterium propionicum is responsible for the breakdown of propionic acid into acetic acid. Hydrogen is released as a acetogenesis, and this hydrogen has damaging effects on the bacteria that are responsible for this process. As a syntrophy, or the coexistence of acetogenic bacteria and autotrophic methane bacteria using hydrogen, is required. Since roughly 70% of the methane produced during the generation of biogas is produced during the reduction of acetates, the phase of acetogenesis represents the efficiency of biogas production; acetates are a crucial intermediary product.

Methanogenesis

By using methanogenic bacteria, methane is produced during this stage. In this stage of the process, methane is created using substrates that are leftovers from earlier stages, such as formate, acetic acid, H2O, methylamine, and dimethyl sulphide, as well as methanol. Despite the fact that only a small number of bacteria can convert acetic acid into methane, heterotrophic methane bacteria convert acetic acid into a significant portion of the CH4 that is produced during the digestion of methane. In this procedure, autotrophic methane bacteria reduce CO2 to generate methane, but only to a lesser extent (only 30%). As H2 is used up, acid bacteria can grow and produce short-chain organic acids during the acidification phase and there are favourable conditions for their development.

Functional Relationships

Species Richness and Disturbance

The hypothesis of intermediate disturbance states that the presence of disturbances of intermediate strength, frequency, magnitude, or duration increases the number of species in a community. Competition and dispersion trade-offs are involved with the storage effect. Disturbance reduces the population of species that are competitive but have poor dispersal, which opens up opportunities for less competitive species with better dispersal. Both types of species can coexist with intermediate disruption because it prevents competitively dominant species from controlling all the resources and allows pioneering species to still discover new areas to colonise. When one species' population growth rate is a nonlinear function of another species' population growth rate, relative nonlinearity is present. These two theoretical models define the prerequisites for the coexistence of species.

The notion of intermediate disturbance is frequently supported by the diversity of stationary creatures, including corals, alpine plants, tropical forest plants, and corals. For terrestrial animals or benthic marine species on soft bottoms, the notion is still debatable and not well established. Even in tropical forests, the intermediate disturbance hypothesis only accounts for a modest percentage of the variation in plant species richness.

The intermediate disturbance theory has a frequently disregarded alternative in spatial heterogeneity. There may be additional successional states and realised niche space of moderate disruption increasing the geographical heterogeneity. In either case, there should be a greater variety of species of intermediate disturbance. A damaged landscape with ant populations serves as an illustration.

The Fall-line Sand hills in central Georgia, in the southeastern United States, have a remarkable diversity of ant species that, at first appearance, seems to support the notion of intermediate disturbance. We discovered that species richness rose in areas that had just little military training-related disturbance. However, regional heterogeneity also reached its apex in response to intermediate disturbance, and species richness grew linearly as a function of spatial heterogeneity. It is possible to explain the association between species richness and disturbance using either one of the two hypotheses (intermediate disturbance or spatial heterogeneity) alone or both hypotheses combined.

Species Richness and Productivity

When production rises, species richness frequently rises at small scales as well before falling as productivity rises further. The frequency of such unimodal species richness-productivity connections, however, is a topic of substantial controversy. According on the selection criteria and research employed, estimates including plant communities range from 20 to 52 percent of studies. Nine people participated in a lively debate of the meta-analyses that have addressed the topic in a recent Ecology forum titled "Evidence and inference: morphologies of species richness-productivity curves." Both species richness and productivity should rise exponentially as long as both feedbacks are positive, since productivity positively influences species richness, which in turn positively affects productivity. A minimum of one feedback connection needs to become zero or reverse itself in order to stop the growth. By putting forth the multivariate productivity-diversity hypothesis, Gross and Cardinale were able to get around this seeming conflict (MPD).

Species Richness-Curves Contingent upon Organism and Environment

As they set out to research unimodal species richness-curves for many taxa, at numerous geographical and temporal scales, in varied biomes, ecologists produced a significant body of literature. Observational studies, experimental techniques, or a mix of the two have all been used in order to accomplish this. Additionally, species diversity is one of many criteria, including the Shannon index and Hurlburt's chance of interspecific encounter. Species richness is either the number of species or the species density, and both of these terms are used to describe species diversity. Similar to how pollution can be monitored along gradients to indicate magnitude, frequency, or a mix of the two, natural stressors like pollution, waves, fire, and salinity can also cause disturbances. Productivity, which also has a wide range of indices or proxies, can affect responses in terms of species richness.

Marine Benthic and Intertidal Organisms

Following ground-breaking research in rocky intertidal zones on the Pacific and Atlantic coasts of North America, the idea of unimodal species richness-curves was developed. In these investigations, predation and herbivory, wave-generated disturbance of substrate and algae populations, and storm-generated disturbance of coral reefs were all investigated. Unimodal species richness-curves in marine benthic and rocky intertidal communities have been tested experimentally and by observation, just like in other taxonomic groups and ecological systems. Additionally, they have taken place at many spatial and environmental scales. Based on his research with coral reefs, Connell proposed the classic intermediate disturbance theory, which postulated that competitive exclusion keeps species richness low in low disturbance conditions because dominant species monopolise resources. Recent studies have shown scale dependence and interactions of productivity and disturbance, along with unimodal patterns of species richness, in benthic marine communities. In Brazil, researchers looked at the larval settling of microbenthic marine communities in oligotrophic and eutrophic bays to determine how the species richness of algal communities on the Swedish coastline varied along both disturbance (waves) and NPP (biomass) gradients. They tested the impacts of disturbance (biomass removal) and nutrient enrichment using a factorial design, and they discovered that the oligotrophic bays, but not the eutrophic ones, showed a dampening of the unimodal relationship.

The position of the mode or the shape of the unimodal species richness-function may change productivity and disturbance interaction. Furthermore, when both bottom-up (production) and top-down (disturbance/consumers) processes are active at the same time, complex interactions may take place. Nutrient enrichment in a marine habitat off the west coast of Sweden had little impact on patterns of unimodal species richness, demonstrating that these relationships are not always obvious.

Terrestrial Plants

The diversity of plant species is a unimodal function of productivity. These authors searched for examples of a monotonic association between plant species richness and production in 1993 but were unable to locate any. The number of unimodal associations has significantly decreased since that time. Three meta-analyses in a row estimated that between 41 and 45 percent, 25 to 25 percent, and 35 percent of studies of vascular plants had unimodal species richness-productivity associations. Whittaker's critical analysis of these species richness-productivity studies revealed that 35 out of 68 research (51.5%) were rejected because they failed to meet the criteria for inclusion. 7 (or 46.7%) of the 15 studies that were eligible were unimodal. Connell, for example, examined a sizable data set of 2504 one-hectare plots (331,567 trees) in wet, moist, and dry tropical forests of Ghana and first promoted them as examples of the intermediate disturbance hypothesis. Unimodal species richness-disturbance relationships have been just as contentious in these tropical rain forests. They used the proportion of stems in a stand that belonged to pioneer species as an indicator of disturbance (range less than 2 percent to more than 90 percent). Even with enormous sample numbers, the unimodal connections were hardly discernible, particularly in moist and wet forests. However, only 12.3% of the variation in species density was described by the unimodal curve, which was best developed in dry tropical forests. Only a negligible 2.8 percent of the variance in the wet forests was explained by it.

Terrestrial Vertebrates

There aren't many examples of correlations between species richness and humpbacks in terrestrial animals, especially terrestrial vertebrates. Most terrestrial animals, including both vertebrates and invertebrates, do not match the three requirements for a unimodal curve of species richness, claim Fuentes and Jaksi. Prior to release resources, the predator or disturbance must lower the densities of competing species. To colonise the resources that have been unleashed, a sizable species pool must be readily available. Third, there needs to be fierce rivalry among the species that are colonising.

Discussion

The last observation speaks to FISH's quantitative components. However, this only applies to homogeneous and equally dispersed samples. The ability to obtain quantitative data over other molecular approaches is a significant benefit. There should be between 30 and 150 microorganisms each section of the microscopic grid. In order to establish statistically significant cell counts, between 10 and 20 regions should be counted. Fluorescence microscopy can count cells; however it can be laborious and time-consuming due to imperfect samples and fluorescent background. The operator's judgement and expertise can affect this procedure. A regulated and mechanised method would be ideal because truth in this situation is subjective. For the purpose of analysing the structure, function, and dynamics of microbial communities, it is normal practise to use numerous molecular approaches concurrently. To assess microbial structure and function, for instance, FISH-SIP, microarrays, and combinations of FISH and micro autoradiography (FISH-MAR) can be utilised. Figure 5 shows how several molecular methods can be utilised to examine the structure, function, and metabolic transformation of microbial communities either alone or jointly. The monitoring techniques that have been used most frequently for microbial community structure analysis include FISH, DGGE, ribosomal inter-genic spacer analysis (RISA), T-RFLP, clone libraries, DGGE, and T-RFLP, with DGGE and T-RFLP being the procedures that are most frequently used in conjunction with other techniques. The most cutting-edge and sophisticated techniques are a few current molecular techniques that have been applied for microbial identification and detection microarray.

Based on phylogenetic distance measurements, Clarke and Warwick have created two biodiversity indices. The path length across the classification tree between randomly selected individuals, averaged across all potential pairs, is used to determine taxonomic diversity for any data collection that contains a list of taxa with phylogenetic or Linnean classification. Consider a sample of benthic macro invertebrates where the majority of the individuals are members of the same order, as opposed to a sample that is abundant in nematodes, plecopterans, trichopterans, ephemeropterans, and dipterans. Although the species richness (or abundance and evenness) of these two samples might be same, the taxonomic diversity scores would be very different. The other comparable metric is taxonomic distinctness, which is calculated by dividing taxonomic diversity by the minimal taxonomic diversity (i.e., all species belong to the same genus). Pure taxonomic relatedness, unaffected by abundance, is represented by the second metric. The fact that taxonomic diversity is independent of sample size, in contrast to other species richness and diversity indices, is an intriguing feature. As far as we are aware, no systematic examination of unimodal species richness data using these alternative biodiversity indices has been done. This study identified positive, negative, and neutral associations across various trophic and taxonomic freshwater groups. There may be value in conducting more research on these topics. The composition of the community is a relevant issue as well. Even if phylogeny's influence is taken into account, communities are not just haphazard assemblages of species. The population composition of fishes, for instance, typically shifts over a pH gradient from acidic blackwaters to alkaline clearwaters. Inconsistent statistical independence is present once more. In circumneutral clearwaters, centrarchids predominate, while cyprinids do. The Felsenstein approach could be used to solve this issue, substituting a cluster diagram (branching order and branch length) for a cladogram and applying an appropriate community similarity (or distance) index.

Conclusion

We aimed to present the background, current novel theories, and complexity of a subject that spans all of ecology in this, admittedly, condensed study of humpbacked species richness-relationships. It has been claimed that unimodal species richness-relationships can be viewed as "contingent rules" because they depend on the organism and the environment, as well as all the restrictions and factors related to scale, environment, and organism. There is the complexity issue, which we have already touched on, in addition to these factors. For instance, the link between species richness and disturbance is influenced by productivity. However, the reality is significantly more complicated because trophic level influences how disturbance and productivity interact. Populations of primary producers (plants and algae) and secondary consumers are constrained in productive environments by competitive interactions (carnivores). Secondary consumers compete for prey while main producers struggle for nutrients, space, light, water, and other resources.

Primary consumers (herbivores), which are insufficient in number to control primary producers, are in turn regulated by secondary consumers. The green-world theory is this. Secondary consumers, on the other hand, are too infrequent in unproductive environments to control main consumers, who are then controlled by resource rivalry. Currently, the primary consumers control the primary producers. Predation pressure, which is likewise a stress, but affects a different group of species, should therefore be largest in the most productive settings, whereas herbivory pressure should be greatest in the least productive ecosystems. There is currently no clear understanding of how these three-way interactions between disturbance, productivity, and trophic level will affect species richness. The most recent research to appear in the field goes beyond merely documenting patterns of species richness and experimentally changing one independent variable at a time. Species richness is influenced by a variety of factors, all of which are interrelated, including disturbance, predation, productivity, succession, environmental gradients, and geographic heterogeneity. At least some of these interactions will be taken into consideration, and spatial scale will also be incorporated, in the most effective models and experiments.

Under anaerobic conditions, anaerobic digestion is a challenging reduction process that involves several metabolic events. By converting biomass into electricity, these digestive techniques help recycle organic waste and lessen the dangers they pose to the environment. Anaerobic digestion goes through several steps, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The many types of microorganisms—fermentative, syntrophic, acetogenic, and methanogenic bacteria—make biogas conceivable. Methanogens, which include hydrogenotrophic and acetotrophic microorganisms as well as substrates including CH3COOH, H2, and CO2, are essential microbes that play a crucial part in the creation of biomethane. Acetoclastic methanogenesis, which involves the direct cleavage of acetate to CH4 and CO2, and hydrogenotrophic methanogenesis, which involves reducing the CO2 with hydrogen gas, are the two mechanisms by which methanogenesis happens. The most prevalent biopolymer on the planet is cellulose, which is also a large component of solid wastes. Anaerobic digestion of the cellulosic waste can produce the primary energy source, methane. Because plant cell walls are highly complicated structures, extensive enzymatic hydrolysis requires a wide range of enzymes. The rumen has large concentrations of three cultivable species of ruminal bacteria that can grow quickly on cellulose: Ruminococcus albus, Ruminococcus flavefaciens, and Fibrobacter succinogenes.

However, simultaneously regulating both disturbance and spatial heterogeneity is very challenging. Thankfully, there is a good chance that the connection between species richness and spatial heterogeneity will be linear. Thus, in their defence of the intermediate disturbance theory, they claimed that one may include spatial heterogeneity into manipulative studies, not as an effect but as a covariate, and that subsequent study should concentrate on the underlying causes. This advice is applicable to all species richness studies, not only those that involve disturbance.

For the purpose of understanding how the microbial populations in anaerobic digesters change over time, many molecular methods, including DGGE and FISH, are being used. DGGE analysis can be used to study microbial populations. This approach is one of the most used fingerprinting methods because it can be used to recover and sequence amplification products, which may be used to describe microbial populations. FISH is just a taxonomic method, which means it is frequently used to determine whether individuals from a particular phylogenetic affiliation are present in the sample. However, it is unable to provide details regarding the role or metabolic characteristics of the microorganisms. When compared to other molecular techniques, the FISH has the benefit of being quantitative, but it can only be used on homogeneous materials with an even distribution of DNA.

Acknowledgement

The author would like to acknowledge his Department of Department of Biology, Berry College, Mount Berry, USA for their support during this work.

Conflict of Interest

The author has no known conflict of interest associated with this paper.

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