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Processes Occurring within the Biofilm

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Abstract

Biological filtration is a complex process consisting of two consecutive stages. The nutrient transport stage, which is influenced by various fluid mechanical and surface-physical factors, represents a precondition for the biochemical process taking place within the biofilm. Biological water filtration is based on the catalyzed decomposition of contaminant molecules with the help of enzymes. This article, in addition to describing the conditions and mode of action typical within this second stage, also aims to explore the practical implications of the established results, including the controllability of biological reactors.

Keywords

biological filtration, Ne-coefficient, pH, redox potential, rH_2

1 Introduction

Artificial drinking water purification (i.e., the process of purifying water in built structures) is mostly carried out by physico-chemical processes. Wastewater treatment is also carried out in built structures in the vast majority of cases. Here too, there are elements of physico-chemical processes, but the purification of wastewater is mainly a biological process [1].

Bank filtration purifies water naturally. It was recognized very early on that the purification of water is not simply a matter of sand filtration, but rather of biological filtration [2].

The inlet water quality of bank filtration and wastewater treatment is different. However, they have in common that the water is purified by essentially biological means. The same microbes are present in both cases [3].

Water contamination is caused by inorganic, but mainly organic molecules mixed in the water. Biological purification of water occurs when microbes break down these molecules, and the new molecules that are produced no longer contaminate the water [4].

Traditionally, indirect parameters are used to monitor whether these degradations occur.

- The COD, BOD parameters indicate the rate of oxygen depletion. If oxygen is depleted, then presumably the molecules are degraded. The oxidation process that takes place is a flameless combustion, which requires a lot of oxygen [5].
- If the number of bacteria in the microscopic image changes or increases, this indicates that energy is available for their reproduction, the source of which can only be decaying molecules. The energy is due to the difference in energy levels between the original molecule and the product molecules [6, 7].
- The biofilm is the habitat of bacteria. It is not just one kind of bacteria, but a community of microbial species "working on the water purification project". Modern PCR technology is used to detect the composition of microbes, but especially their changes. The result is a bar-coded graph showing the diversity of microorganisms within the community. The thicker line indicates the larger group. From the images taken at different times, we can see the changes and deduce how the contaminant molecules are being degraded. [8]
- It is also common to study the gas composition of the air above open biological reactors. The appearance of certain molecules, mostly CO_2, indicates that the water is being purified. [9]
- In wastewater treatment, in addition to the quality of the water, the degree of sludge depletion can also be tested. This is indicated by the difference in the calorific value of the sludge measured at the start and end points of the treatment process. The decrease in the calorific value of raw sludge and surplus sludge indicates that the organic molecules causing the

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pollution have decomposed. By analogy, the change in calorific value can also be considered as a measure of water purification. [8]

Much less attention is nowadays paid to the inherent essence of biological water purification, the conditions under which molecules are degraded. This direct phenomenon of water purification does not receive sufficient attention.

Two things are necessary for the degradation of a polluting molecule. The first step is to achieve biofilm penetration, which is driven by a difference of concentration, by diffusion. The similarity criterion for the transport process is the Pe value, which is determined by the equivalent particle diameter of the biofilm-etching surface, the relative velocity of the water and the biofilm-bearing surface, and the diffusion coefficient of the contaminant molecule in the water-soluble state. By varying the Pe value, it is possible to improve the nutrient supply to the biofilm and thus increase the degradation efficiency [10, 11].

Degradation of the molecule must occur within the biofilm. Enzyme catalysis is the underlying mechanism of this complex biochemical process. The similarity criterion for this second step is the Ne-factor, which can be given as a function of pH and rH_2. The pH dependence of microbiological processes has been shown in many studies and is generally accepted as a parameter. The significance of the dimensionless redox potential, or rH_2, is only rarely foregrounded and is not a widely used indicator [12].

In the following, in a somewhat unconventional way, going back to the original reasons and ideas, the conditions of molecular degradation within the biofilm will be discussed. We do this primarily to bring new considerations to bear on the operation of biological reactors, as we see in [13].

2 Problem definition

During the time of cholera and plague pandemics in England, Snow[1] established that both diseases were spread via drinking water [14]. While researching into anthrax, Pasteur[2] sought to weaken anthrax bacteria by modifying their environmental conditions, such as temperature, nutrient supply, and exposure to air [14]. Vincent[3], a follower of Pasteur's work, went further and drew a "map" of the preferred living conditions (or "climatic" conditions) of certain disease-causing microbes. He found that the examined pathogenic microbes only remain viable within a well-defined section in a plane determined by the dimensionless variables pH and rH_2, [15, 16]. His views on how pathogens could and should be fought were summarized in the following theorem: "Deprive the disease from its vital conditions, and the disease shall cease to exist". In practice, this means that the pH – rH_2 environment must be modified to an extent where pathogenic microbes become non-viable (see Fig. 1) [17, 18].

However, biological water filtration is not aimed at fighting microorganisms. On the contrary, it relies upon sufficient bacterial growth: the more contaminated the water, the more 'workforce', i.e., the more bacteria, will be required for its purification.

Bearing this in mind, by reversing Vincent's logic we can conclude that bacterial activity can be promoted by creating and maintaining an optimized 'climatic' environment for the involved microbes. Like all organisms, the non-pathogenic bacteria performing the actual decomposition of nutrients have their own ideal environment, the area of which can be specified in the pH – rH_2 plane. According to our theory, in order to facilitate an effective biological water purification, the relevant environmental factors must be fine-tuned for the bacteria we are seeking to employ. But what are these (necessary and sufficient) conditions exactly? This is what we are aiming to specify in the following sections of this paper.

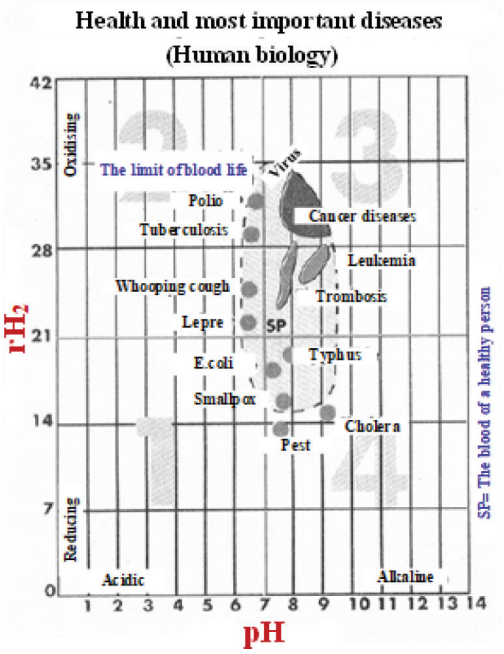


Fig. 1 Vincent's bioelectronic diagram of diseases [17, 18]

1 John Snow (1813–1858) British Physician, Epidemiologist and Anaesthesiologist

2 Louis Pasteur (1822–1895) French Chemist and Microbiologist

3 Louis-Claude Vincent (1906–1988) French water research engineer, father of bioelectronics

3 Background

Biological water filtration relies on an active involvement of microorganisms responsible for the decomposition and transformation of contaminant molecules. The new water-borne compounds generated through this process are completely harmless [19, 20].

The modelling of biological nutrient decomposition has shown that the process in question consists of two consecutive stages. Although bacteria show certain movement within the biofilm (i.e., the living area formed on the biofilm carrier media such as sand, floccule, activated carbon, membranes, or roots), they tend not to change their places or alter their microenvironment substantially, apart from changing their physical location along with the eventual expansion of the biofilm. This means that the nutrients to be decomposed must be transported to the bacteria via a combination of convection and diffusion. The similarity criterion of this process is represented by the Pe-number. This physical sub-process, which fulfils a key precondition for the actual filtration stage, takes place outside the biofilm, within the extracellular body of water.

All molecules, including the ones that contaminate water, can generally be described as inherently stable formations, where their stability is primarily secured by a given amount of activation energy. In order for the contaminant molecules to be decomposed, an activation energy of $\Delta E_{\text{activation}}$ is required. Nevertheless, should certain enzymes be involved in the decomposition process, the level of required activation energy decreases, due to which contaminant molecules are decomposed more easily and the formation of new, smaller molecules (a.k.a. decomposition products) is catalyzed.

As the combined energy level of the generated products is lower than the energy level present prior to the decomposition, the difference will be released (ΔE, see Fig. 2). The thusly generated energy, i.e., the difference in the energy level of the substrate and the combined energy level of all decomposition products, will then be utilized by the bacteria for sustaining and reproducing themselves.

As shown in Fig. 2, the fluid at the bottom of a tall glass is fairly stable, whilst it is easily spilt from a shallow vessel. Likewise, enzyme catalysis must be regarded as a factor reducing 'the height of the glass', which here actually means reducing the level of required activation energy, enabling the molecule to decompose more easily. Nevertheless, enzymes leave the amount of energy released through the reaction (ΔE) basically unaffected.

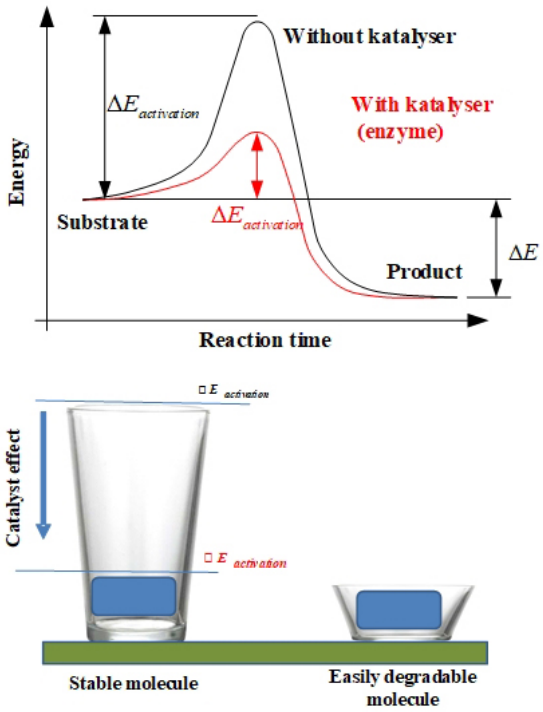


Fig. 2 Representations of the enzyme catalysis process

This characteristic of the enzymes is also utilized by the natural environment while it performs its own water filtration processes.

Enzymes are large molecules as well. They are not involved in the decomposition process itself and they do not undergo any permanent change – they merely facilitate the transfer of an atom or radical from one place to another. In other words, they serve as catalysts [21]. In the natural environment several types of enzymes can be found. However, the decomposition of a given molecule can only be catalyzed by an enzyme whose 'pattern' is identical with that of the molecule. This phenomenon is described by the Michaelis-Menten[4] kinetics model (known for over 100 years), which supplies an equation relating reaction rate to the concentration of a substrate [22].

All bacteria are single-celled organisms, consisting mainly of bacterial proteins, a.k.a. enzyme-proteins. Microbes, after breaking down substrate molecules, utilize the released energy for sustaining themselves, for growing and reproducing. The laws of reproduction are also described by a kind of kinetics, the Monod[5] kinetics. The rate of multiplication also depends on the substrate concentration.

4 Leonor Michaelis (1875–1949) German Biochemist; Maud Menten (1879-1960) Canadian Medical Researcher

5 Jacques Monod (1910–1976) French microbiologist and genetist, Nobel Prize in Medicine (1965)

The substrate itself is commonly regarded as food for bacteria. Nevertheless, this definition does not prove completely accurate, regarding the fact the bacteria are not directly involved in the decomposition of nutrients – it is only the enzymes in their cells that act as catalysts. The increase in the number of microbes due to cell division can, at the same time, be regarded as an 'inexhaustible source of new workforce'.

The actual decomposition of contaminant molecules takes place within the biofilm, where one single type of molecule can be decomposed by different types of bacteria. From among the countless types of bacteria present in the biofilm, it tends to be the ones who find the prevailing environmental conditions most favorable that reproduce more actively. The 'ideal working conditions' for any bacteria are, typically, jointly determined by the pH (acidity or basicity), rH_2 (nondimensional redox potential) and T (temperature) parameters of the environment.

In an oxic environment the decomposition products generated through the biochemical reaction generally include carbon dioxide and water, whereas in an anoxic (anaerobic) environment they tend to include carbon dioxide and methane. None of the decomposition products have a harmful effect on the filtered water as the generated carbon dioxide and methane either escape into the atmosphere or, alternatively, they can be captured and utilized as biogas.

4 Properties of the pH - rH_2 diagram

The reactions occurring in the water can basically be twofold: acid-base or oxidation-reduction reactions. Acid-base reactions are based on proton-transfer, whereas redox reactions rely on electron-transfer. The properties of both processes are illustrated in more detail in Table 1.

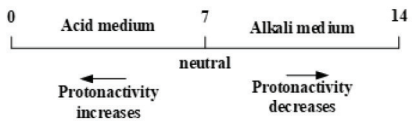
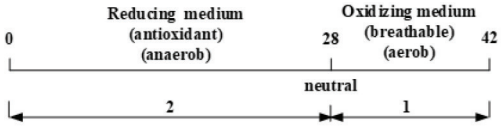
The pH and rH_2 parameters are not independent from one another. The relationship between them can be described by means of the following equation [23, 24]:

$$rH_2 = \frac{2FE_h}{RT\ln 10} + 2\text{pH}\,, \tag{1}$$

where

- F Faraday constant,
- E_h redox potential for the standard hydrogen electrode,
- R universal gas constant,
- T absolute temperature.

Table 1 Interpretation of the pH and rH_2 parameters

	Acid-alkali reactions	Redoxi reactions
Reaction	proton transmitting $H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$ (other way $H^+ + OH^-$)	electron transmitting $H_2O + H_2O \rightleftharpoons 2H_2O_2$
Chemical poise	$k_w = [H^+]*[OH^-] = 10^{-14}$	$k_e = [H_2]^2*[O_2] = 10^{-84}$
Chemically clean water	$[H^+] = [OH^-] = 10^{-7}$, so $k_w = [H^+]^2 = 10^{-14}$	$[H_2] = 2[O_2] = 10^{-28}$, so $k_e = {}^1/_2[H_2]^3 = 10^{-84}$
Neutral point	$\sqrt{10^{-14}} = 10^{-7}$	$\sqrt[3]{10^{-84}} = 10^{-28}$ (exactly $\sqrt[3]{2\times10^{-28}} = 1.26\times10^{-84}$)
Definition	$pH = \log\frac{1}{[H^+]} = -\log[H^+] = -\log\frac{[H^+]}{1}\frac{mol/L}{mol/L}$ pH = power of Hydrogen	$rH_2 = \log\frac{1}{[H_2]} = -\log[H_2] = -\log\frac{[H_2]}{1}\frac{mol/L}{mol/L}$ rH = *reduction* of Hydrogen ORP = Oxidation Reduction Power
Discover	Sorensen, S. P. L. (1868–1939), danish chemist, 1913	Clark, W. M. (1884–1964), american biochemist, 1920
Chemically clean water	$pH = 7$	$rH_2 = 28$
Scale	Symmetrical scale 	Unsymmetrical scale 
Comment	Acid: proton delivery materials pH decreases with acid addition Alkali: proton absorbing materials pH increases with acid addition	$rH_2 = 0$ H_2 can get rid of $rH_2 = 42$ O_2 can get rid of $rH_2 = 0...42$ the limit of thermodinamic stability of water, there exist no water outside this interval

The dimensionless formula of the redox potential is also known as the Nernst-coefficient.

$$\mathrm{Ne} = \frac{2FE_h}{RT\ln 10} \tag{2}$$

With this substitution the pH – rH_2 correlation can be simplified to the following form:

$$rH_2 = \mathrm{Ne} + 2\mathrm{pH}\,. \tag{3}$$

Let us note that after rearranging the formula, the redox potential

$$E_h = \frac{RT\ln 10}{2F}rH_2 - \frac{RT\ln 10}{F}\mathrm{pH} \tag{4}$$

can be determined as the algebraic sum of two addends, where the first addend depends on the electron exchange between dissolved solids and water whereas the second addend exclusively depends on the proton exchange.

In the plane spanned by the pH – rH_2 variables, a.k.a. the Vincent diagram, all functions for Ne = constant are linear. Relying on the interpretations of Table 1, this allows four the determination of four distinct sections (see Fig. 3), each of which can be associated with various concepts known from the field of bioelectronics.

These include, among others, the notion of vital water associated with a healthy way of life, the notion of harmless water and other related connotations known from the field of natural healing.

Nevertheless, let us ignore the broader bioelectronic context for the time being and solely focus our attention on the plane spanned by the variables.

5 The role of the Ne-coefficient in the nutrient decomposition function

The function obtained through the application of dimensional analysis takes the following form [25, 26]:

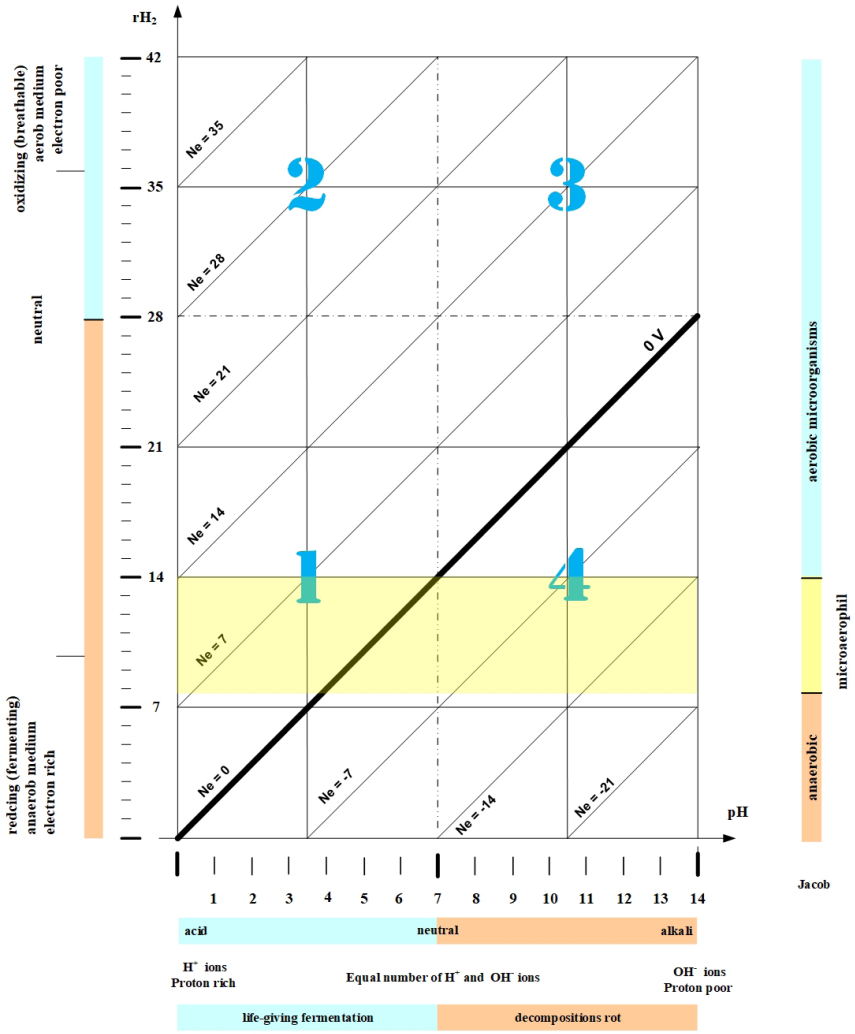


Fig. 3 The Vincent-diagram and its sections

$$\Delta S = \mu(\mathrm{Pe})\frac{1}{\mathrm{Pe}} S\mathrm{Ne}^{1/3}, \quad (5)$$

where:

- ΔS Substrate degradation rate,
- μ Filtration coefficient,
- Pe Pe-number (Péclet number),
- S Substrate concentration,
- Ne Ne-coefficient (Nernst-coefficient).

Putting the derivation details aside, let us now focus on the Ne-coefficient, whose heuristically chosen exponent is 1/3. The formula used in the derivation of the dimensional analysis.

$$\mathrm{Ne}^* = \frac{FE_h}{RT} \quad (6)$$

only differs from function (Eq. (2)) in one constant.

In function (Eq. (5)) the Ne-coefficient represents the 'climatic conditions' inside the biofilm, which can also have a negative value. Consequently, the cube root extraction may here require some further explanation. The three roots include some complex roots as well. However, it is only the roots with positive real parts that bear a meaning from a physical point of view as nutrient decomposition can exclusively be interpreted as positive. Therefore, the function values of roots derived from negative numbers will only be half as high, as shown in the representation plotted for the Vincent diagram below (Fig. 4).

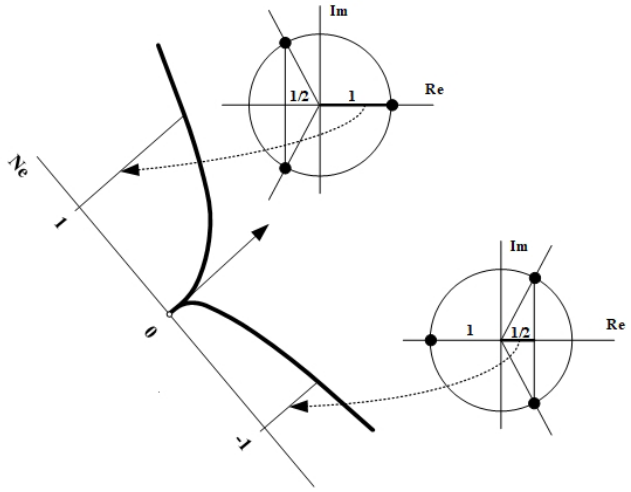


Fig. 4 Cube root of the Ne-coefficient

In the north-west direction the value of the Ne-coefficient is positive, whereas to the southeast it is negative.

6 Representation of the measurement results

After reviewing the interpretations and mathematical aspects of our research, let us take a closer look at the actual measured results.

For the purposes of this research, various measurement points were established at different operating biological reactors (see Table 2). The individual reaction zones were, at the same time, meant to identify the microbial community generally existing there.

Table 2 Results from performed measurements and other sources

	Case	pH [-]	t [°C]	T [K]	F [C /mol] [A s /mol]	R [J/(mol K)] [V A s/ (mol K)]	ORP [mV]	korr(t) [mV]	Eh [mV]	[V]	Ne [-]	rH_2 [-]	Date
1	Danube river at Dunakutató Göd	8,00	10	283	9,65E+04	8,31	190	214	404	0,40	14,4	30,4	
2a	Kisoroszi well 1 at the riverbank	8,00	21,6	294,6	9,65E+04	8,31	84	202	286	0,29	9,8	25,8	2010.07.05
2b	Csepel well 11 at the riverbank	7,80	18,5	291,5	9,65E+04	8,31	128	205	333	0,33	11,5	27,1	2010.07.06
3a	Sopron wwtp anoxic basin	7,53	21,1	294,1	9,65E+04	8,31	-37	203	166	0,17	5,7	20,7	2018.07.01
3b	Sopron wwtp oxic basin	7,10	21,3	294,3	9,65E+04	8,31	-17	202	186	0,19	6,4	20,6	2018.07.01
4a	Tentative mezophil fermenter min.	7,60	39	312	9,65E+04	8,31	-450	185	-265	-0,27	-8,6	6,6	
4a	Tentative mezophil fermenter max.	7,60	39	312	9,65E+04	8,31	-550	185	-365	-0,37	-11,8	3,4	
4b	Tentative thermophil fermenter min.	7,60	55	328	9,65E+04	8,31	-550	168	-382	-0,38	-11,7	3,5	
4b	Tentative thermophil fermenter max.	7,60	55	328	9,65E+04	8,31	-600	168	-432	-0,43	-13,3	1,9	

In the cases involved, the water body of the river Danube was used to represent a model for self-cleaning rivers (1), whereas the bank sections at the islands of Csepel and Szentendre (2a, 2b) served as models for bank filtration. The measurements performed in the oxic and anoxic tanks at Sopron are meant to illustrate the conditions prevailing in activated sludge tanks (3a, 3b) and, last but not least, the data for the mesophilic and thermophilic digesters of FCSM[6], the capital's sewage works (4a, 4b), were gathered from various recent publications [27, 28].

In Table 2 the values listed in columns pH and ORP are measured values, while F and R are physical constants. The data shown in all other columns represent calculated values.

Although most of the applied calculations appear to be straightforward, the conversion of the redox potential might require some further explanation. The instrument used to establish the relevant oxidation-reduction potentials (ORP) measures the potentials between the redox system (in our case, the reaction zone) and the probe. Nevertheless, for the calculation of the Ne and rH_2 variables, the E_h values need to be established. The correction rate depends on the prevailing temperature [15].

$$korr(t) = 223.9 - 1.01t\,, \tag{7}$$

which results in the correction formula

$$E_h = ORP + korr(t)\,. \tag{8}$$

The Ne-coefficient can eventually be calculated with the help of formula (Eq. (2)), and the rH_2 values on the basis of formula (Eq. (3)), respectively. Relying on the findings presented in Table 2, we can further plot a graph of the relationships between the different reaction zones (see Fig. 5).

Based on the above representation, the following general conclusions can be drawn:

- Bankfiltration, activated sludge-based wastewater treatment and sewage sludge digestion equally take place in the $rH_2 < 28$ domain.
- Self-cleaning in rivers (in our case in the river Danube) occurs under aerobic conditions ($rH_2 > 28$). Substrate decomposition rates appear to be the highest with this method, which means that the Ne-coefficient and the cube root value extracted from it are also the highest here.

6 Budapest Sewage Works

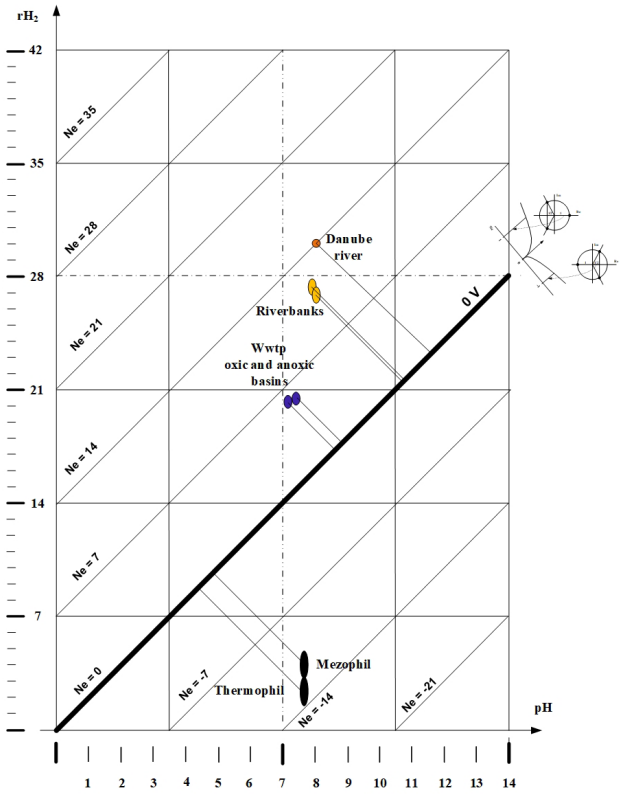


Fig. 5 Measurements performed

- The pH values prevailing in the different reaction zones hardly vary. At the same time, there is a significant variation in the rH_2 values. Based on this, we can risk the conclusion that the role of the dimensionless rH_2 coefficient – unduly neglected so far – must be far more significant from the point of view of understanding bacterial diversity than we had ever dared to presume.
- Sewage sludge digestion is performed under strictly anaerobic, anoxic circumstances, in the Ne range of $Ne < 0$, as shown on lines (4a and 4b) of Table 2. According to these results, thermophilic digestion appears to be far more effective than mesophilic digestion. As we can see from Fig. 5, the $Ne_{thermophilic}$ intercept is demonstratively longer than the distance to $Ne_{mesophilic}$. These findings have been unequivocally verified by the operator's experience as well.

However, according to the cube root extraction the decomposition rates are only half of those established under oxic, $Ne > 0$ conditions. This result is also supported by the prolonged bacterial reproduction time (ca. twice as long) observed in the case of the digestion method [29, 30].

- For the time being, no direct experimental proof is available to support the assumption that, according to formula (Eq. (5)), the substrate decomposition rate equals to 0 if Ne = 0. The slope of the function curve on both sides is ∞ for Ne = 0. The 'transition' section appears to be rather narrow, and an explanation for its existence might be supplied by Jacob's observation. Jacob[7] classified bacteria according to rH_2 values, thus establishing three distinct groups: anaerobic micro-organisms, aerobic micro-organisms and, between the two other groups, micro-aerophilic microbes. The relevant sections are also represented in Fig. 3.

  Although the sections obtained through Jacob's classification and the ones based on the functions of the Ne-coefficient do not overlap in the range $7 < pH < 8$, their nature shows high similarity. Nevertheless, the two transition sections are not exactly situated at the same points.

  Also, there seems to occur a contradiction regarding the characterization of bacteria in relation to O_2, namely that the neutral point for rH_2 is at 28. Along this logic, aerobic micro-organisms must be present in the $rH_2 > 28$ range only, whereas according to Jacob their range starts at $rH_2 > 14$. This can be explained by the excessively adaptive metabolic processes of certain bacteria, which enable them to function both as hydrogen donors and hydrogen acceptors and react with a wider range of partner reactants. In other words, during the decomposition of the same organic molecule, oxygen, nitrate or even sulphate can serve as acceptors before fermentation would commence.
- There appears to be hardly any difference in the 'climatic conditions' prevailing in oxic and anoxic activated sludge tanks. The points corresponding with the values represented on lines 3a and 3b of Table 2 are almost identical, suggesting that nitrifying and denitrifying bacteria tend to thrive under the same climatic conditions. In reality, it can hardly be the case. In such a complex system the rH_2 coefficient does not only depend on the presence or absence of oxygen, but also on the concentration of oxidized and reduced species of various ions and compounds. Oxygen merely represents one of the many factors that bear an influence on the process.

  A method for enhancing the efficiency of denitrification (put forward in [31]) suggests that non-aerated reaction zones should be covered by means of floating panels, which are meant to prevent air from dissolving into the fluid. Such an intervention, translated into the language of our own concept, would merely alter the climatic conditions prevailing in the reaction zone, leaving nutrient transport processes (represented by the Pe-number) completely unaffected. Eventually, according to the biological filtration theory, covering the water surface would only prove effective and sensible if it could generate an increase in the Ne-coefficient. It is, therefore, highly recommendable to establish sufficient measurement data either to support or disprove this theory.
- Fig. 5 represents a high level of similarity between the results obtained for the bank sections at the Islands of Szentendre and Csepel. As we can see from the graph, the points determined by the values on lines 2a and 2b of the chart are almost identical.

  Earlier on, before actually performing the measurements for the purposes of this study, there appeared to be a substantial difference in the efficiency of bank filtration at the Szentendre and Csepel bank sections (north and south of Budapest, respectively) as the well water extracted at Csepel would require further treatment prior to utilization. According to a study researching into the aforementioned differences between the two bank sections [25], the increased iron-manganese concentration of the well-water at Csepel was due to a deterioration in the oxic conditions of the relevant bank section. As oxygen and iron electrodes are highly similar, Iron(III) starts to reduce to Iron(II) once the amount of available oxygen decreases, even to the slightest degree.

  Any such decrease in the oxygen level can be generated by a wide range of factors, e.g., by a dead zone created under a navigation spur that reaches over the riverbed or by wastewater channeled into the river without being treated appropriately, among others. As our measurements were performed in this river section after the capital's central wastewater treatment plant (BKSZTT[8]) had been put into operation, no untreated wastewater was channeled into the river Danube at the time this experiment took place. We have found that the river was/is no longer running an 'intensified self-cleaning program' and the

7 Francois Jacob (1920–2013) French Biochemist, Geneticist, Nobel Prize Winner in Medicine (1965)

8 Central Wastewater Treatment Plant of Budapest

water body along the bank was/in no longer in danger of oxygen shortage. That is why the results do not indicate any difference between the two bank sections now, from which we can conclude that the operation of drinking water treatment plants at Csepel and Ráckeve now proves superfluous (or will do so, in the near future).

Naturally, since the central WWTP had been put into permanent service, it is not possible to 'restore' the original conditions (i.e., high amounts of untreated wastewater channeled into the river Danube at Budapest) just for the purposes of further comparative research. Nevertheless, many of the dead zones formed under navigation spurs still exist, which enables the measurement and comparison of conditions with uninhibited and inhibited oxygen flow at different bank sections. Should the difference caused by the oxygen flow be supported by further such measurements, these could – at the same time – serve as proof for the viability of the Vincent-diagram.

7 Why is it essential to keep microbiological laboratory samples cool during transport?

First, let us review an experienced laboratory manager's sentiments on this question:

'When performing microbiological research, we strive to define bacterial count as well as colony count. The reproduction features of bacteria in flowing water (pipelines) tend to differ considerably from those in still water (sample containers). The major aim of sampling is to determine momentary organism counts in the pipeline. The reproduction of these organisms and, in effect, the bacterial count is influenced by sample processing times as well as by sample storage (or 'breeding') temperatures. Once we have taken a sample of flowing water and reduced its temperature, we actually inhibit the reproduction of the organisms it contains. Were we to deviate from this standard procedure, our research results would be impaired and not represent a true picture of water quality prevailing at the given sampling point. This we can support through manifold proof: variations in the measured data based on differing colony counts, storage times and storage temperatures.'

But does this view provide us with a full and valid explanation for the original question or does it require further expansion?

For our reasoning we will rely on the Ne-formula (Eq. (2)) as a starting point, which contains absolute temperature in its denominator. Whenever we reduce the temperature of a sample, we practically increase the Ne-coefficient. The higher the Nernst-coefficient, the 'livelier' the bacteria. There is no organized flow in the sample, which in effect means that there is no convective nutrient transport and the amount of substrate in the sample is hardly sufficient. Under such circumstances, microbes would definitely 'starve to death'. Therefore, if they are meant to grow and reproduce, they must be provided with a more favorable climate. This means that cooling in fact helps keeping micro-organisms alive rather than threatening them -and we do want to keep them alive to be able to perform laboratory tests on them.

In the laboratory, the culture media is inoculated with the sample in a Petri dish. The composition 'pattern' of the culture media must be identical with the enzyme pattern of microbes we aim to identify. As there is no flow within the Petri dish, either the culturing procedure is performed at temperatures between 22 °C and 37 °C. (At a higher temperature the diffusion coefficient would increase.) Through the thusly generated diffusion the nutrient molecules will be transported to the microbes, following which the substrate molecules will be decomposed and the microbes can use the released energy to reproduce themselves. The culture will become 'visible', and the culture count will constitute the test result. Should the sample not contain any microbes matching the pattern of the culture media, they will not be able to reproduce and, consequently, the results of microbiological tests performed on them will be negative – which is, all in all, a positive (!) outcome from the point of view of the operator.

8 Controlling biological filtration processes

Biological water filtration relies upon the same method as the stabilization of sewage sludge by means of digestion, although the two processes require different conditions. Nevertheless, our expectations regarding the outcome of both processes are the same: maximum efficiency, combined with reliable controllability [32, 33].

In order to develop the relevant control technology, we need to be able to establish various parameters through measurement. But what parameters should we focus on? Before supplying an accurate answer to this question, it might prove expedient to explore some of the required conditions, however trivial they might appear.

From a measuring technological point of view, water quality measurements do not tend to qualify as simple measuring methods. They can be divided into three distinct groups:

- Certain water quality parameters are obtained by means of measuring probes. With this method, the measuring sensors are installed directly within the reaction zones, and the transmitted measurement data can be received via the SCADA system. The parameters generally measured by this means include pH, ORP, temperature, dissolved oxygen, conductivity, turbidity, and residual chlorine.
- Another group of applied measuring technologies is represented by water quality analyzers, which perform transmitted measurements as well. The instruments used with this type of measurements require constant attention and specialized knowledge. They generally facilitate automated sampling and require the application of reagents (unless we use especially selective electrodes). Due to the complexity of these measuring instruments, such measurements can only be performed on a periodical basis. The analyzers are mostly used to establish concentration-type quantities, such as PO_4^{3-}, NH_4^+-N, NO_3^--N, (TOC).
- The data obtained by means of laboratory measurements, on the other hand, are not directly received via process control systems, i.e., they are not transmitted measurements. In order to perform these measurements, samples must be collected. The types of the parameters only measurable in laboratories typically include: COD, BOD, TOC, various concentrations and compositions, the energy content of sludges, the specific surface is about biofilm media, diffusion coefficients, microbiological and microscopical measurements, etc.

Considering the instrumentation of wastewater plants, we must conclude that it tends to be the results of the involved biochemical processes, various output parameters as well as the concentration of generated compounds that are extensively studied. At the same time, significantly less attention is paid to analyzing key process-related parameters such as nutrient transport to the microbiota or the climatic conditions within a reaction zone.

Output results usually represent effects or consequences, and they are generally measured by means of slow feature analysis. From a control technological point of view, we must point out that the transition functions for these values tendentially involve delays and possible timeouts, which renders them less applicable for control purposes.

On the other hand, the parameters characterizing the nutrient transport process and 'working conditions' of the bacteria represent causes that do not involve any delaying factors and they can, therefore, be measured by means of rapid analysis probes.

Bearing all this in mind, we can specify the basic requirements for control technical systems monitoring the operation of biological reactors. First of all, the sets of monitors controlled by the dispatchers must include a screen that displays the logistic conditions of nutrient transport, i.e., one that monitors eventual changes in the Pe-number. Secondly, the 'working conditions' of the bacteria must be projected by means of a Vincent diagram. Latter strictly requires the monitoring of pH values, temperatures, and redox potentials within the reaction zones, which can be performed via measuring probes[9]. Today, unfortunately, these methods of data projection – however convincingly supported by relevant theories – are still missing from the monitors of SCADA systems.

9 Conclusions

In 1854, in his inaugural address at the University of Lille, Pasteur emphasized that "without theory, practice is but routine born of habit. Theory alone can bring forth and develop the spirit of invention." [34]. A team of specialist in the Budapest Sewage Works (FCSM) appear to have reached a similar conclusion: "… our current understanding of the phenomena is of a decidedly empirical nature and cannot hence provide an adequate explanation" [27].

The above quotes clearly direct our attention the necessity for new theories and models to be constantly developed – one by focusing on their import, the other by admitting the lack thereof. The axiomatically founded theory of biological filtration, which examines bank filtration, wastewater treatment and digestion from the same point of view, represents a good example of model development based on 'cross-border considerations'. This theory also emphasizes that both disinfection and water filtration tend to rely on the same phenomenon – namely the behavior of microbes –, and they merely differ in their intent to restrict or stimulate bacterial activity, respectively.

Biological drinking water purification and wastewater treatment are two related fields of science that have, up to this date, been treated, studied, and taught separately. This article clearly shows that the secret of achieving further

9 Instead of ORP probes, rH_2 probes equipped with a correction function would be preferable.

results in both fields lies in 'joining their forces'. Any theory can only be correct if it is able to supply adequate answers to all arising questions, may these relate to the biological self-cleaning of rivers or to the cooling needs for transporting and storing laboratory samples. Likewise, it is only possible to find a satisfactory and reliable solution to the problem of safely controlling biological reaction zones if we are ready to think at a practical as well as a theoretical level.

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