Environmental Biochemistry — A New Approach for Teaching the Cycles of the Elements

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Introduction

The biochemical pathways developed by many organisms for satisfying their nutrient and energy demands, often contribute to the recycling of elements in an ecosystem. The importance of such processes in the nature were early stressed by Winogradsky, Beijerinck and Van Niel (see refs 1, 2) who demonstrated that special groups of microorganisms normally participate in the redox reactions which occur in different habitats of the earth. The carbon, nitrogen and sulfur cycles were proposed for describing the most important processes involved in the cyclic turnover of the elements in nature.[1–3]

Transformations in Nature

The complex redox equilibrium of the elements established in any habitat results from the interaction of two components, one originating in physicochemical reactions and the other from biological processes. Although the first can sometimes exclude the second, usually both are interactive and either can induce a corresponding modification in the development of the other.

As in any redox process, matter flows from an oxidized or reduced state towards the opposite, depending on the initial conditions provided by the environment. Therefore, a reduced ecosystem corresponds to a medium rich in electrons, which are easily available to microorganisms for reducing the oxidized 'energy transducer' of cells (NAD^+). Normally anaerobic conditions are representative of such media. Conversely, aerobic conditions are typical of an oxidized habitat, poor in electrons. However, habitats with different redox degrees are found in which oxygen is absent: in these conditions, organic or inorganic compounds may be used as electron acceptors. If oxygen is absent but an electron acceptor is present, the redox process that takes place in cells is called 'anaerobic respiration'.[4–6] There is appreciable difference between the processes which could be expected in aerobic/anaerobic habitats or oxidized/reduced habitats.

The former are examples of biochemical pathways employed by organisms for delivering to electron acceptors the excess of electrons produced by their own metabolic pathways. Other pathways may be used by microorganisms in aerobic, anaerobic, oxidized or reduced media, for obtaining energy. In these mechanisms the intermediary molecule is NADH. Therefore the couple, $NADH/NAD^+$, represents one of the most important biological redox system that allows the organisms to obtain useful energy from the environment. A close relationship between the extracellular physicochemical characteristics of the environment and the metabolic reactions displayed by the microorganisms is observed. Highly reduced media should allow the development of microorganisms which should be able to displace the redox equilibrium towards NAD^+. This is the case in fermentations, where a suitable substrate $(CH_2O)_m$ is consumed as carbon and energy source, producing components more oxidized (CO_2) or reduced (fermentation products, $(CH_2O)_n$) depending on the particular pathways employed by the organisms. If in the later situation it is incorporated an electron acceptor, the equilibrium will tend to NAD^+ through an electron chain, inducing anaerobic respiration. For this reason, we decided to separate the redox characteristics of the medium and the availability of oxygen, as two independent variables, as shown in Figure 1. Furthermore, if, in the above conditions and in the absence of an electron acceptor, the organisms assimilate CO_2, biosynthesis becomes unfavorable unless another electron source is present. This is the case of photoautotrophic bacteria.[7,8] A similar situation may be observed under aerobic conditions when NADH is oxidized via the respiratory chain and NADPH is also consumed for carbon assimilation by using the Calvin cycle.[9] This occurs in chemolithotrophic bacteria.[10] From this point of view, biological matter represents an intermediate component between the completely oxidized and reduced nutrient and energy source, independently of the presence or absence of oxygen.

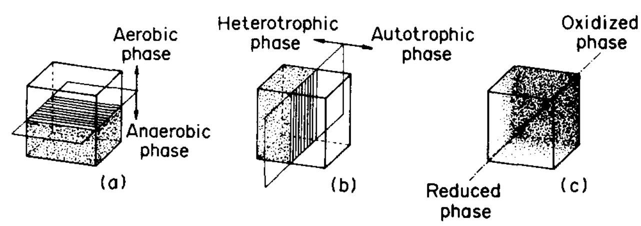

Figure 1 Cube division into trophic and environmental regions

According to the importance attributed to the redox couple $NADH/NAD^+$, we decided to incorporate this variable as a third dimension along with the two dimensional representation of the cycles of the elements (carbon, nitrogen and sulfur)[1,2] but in terms of the redox degree of the environment (oxidized/reduced phases). We suggest the representation of these cycles as a cube which is divided into many regions depending on the environmental characteristics such as: aerobic and anaerobic phases (Fig 1*a*), reduced and oxidized phases (Fig 1*c*), and two physiological regions according to the capacity for assimilation of carbon source, autotrophic and heterotrophic regions (Fig 1*b*). This representation may be used to define the main metabolic feature of any organism depending on its position in the subspace of the cube. Inside the cube, aerobic and anaerobic phases should

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clearly be separated as should the assimilation processes of the carbon source.

Although further sophistication may be included by assigning to the cube different degrees of tolerance to oxygen or facultative autotrophic metabolism, we have not considered these. However, we have considered the degrees of reduction of the environment and matter, in the sense mentioned above, for better understanding the relationship between environmental conditions and the most important metabolisms which microorganisms may display in those habitats.

The Carbon Cycle

Including a third dimension into the well known diagram of the carbon cycle[1,2] we obtained Fig 2 which shows the main processes by which carbon is oxidized or reduced according to the environmental conditions. In Fig 2, the reduction of carbon dioxide could take place in an aerobic (upper) and an anaerobic (lower) phase. In aerobic conditions, carbon dioxide reduction takes place in plants, algae or chemolithotrophic bacteria. These processes can be found in a special region of the cube designated as I. In region II, anaerobic assimilation of carbon dioxide may be identified as the zone where photoautotrophic bacteria would be found. In both regions (I and II), the most important product of synthesis is organic matter designated as $(CH_2O)_m$, which can be set on an oblique plane containing CO_2 but in a different vertical plane according to the degree of reduction of the organic compounds (ie between CH_4 and CO_2).

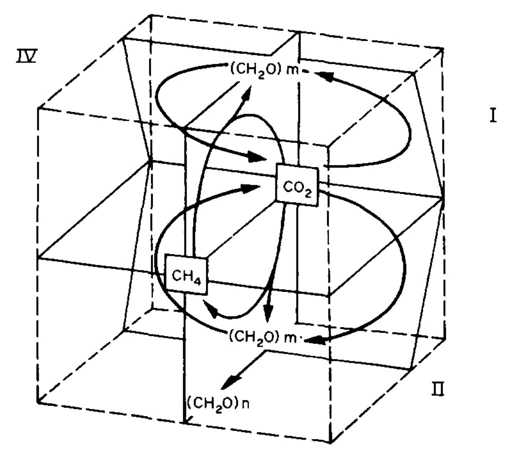

Figure 2 Three dimensional arrangement of the carbon cycle

Heterotrophic fermentation and respiration, are drawn by representing the oxidative processes which occur during anaerobic (region III) or aerobic (region IV) assimilation of organic compounds respectively. In the anaerobic fermentation processes, the organic substrate induces not only the evolution of the CO_2, but also the reduction of an internal organic electron-acceptor, which constitutes the fermentation products. The latter process, was considered in Fig 2 in the pathway described as $(CH_2O)_m \rightarrow (CH_2O)_n$, where $(CH_2O)_n$ designates the reduced organic compound resulting from anaerobic fermentation.

This representation records the fact that in any biological reaction involved in microbial growth, a redox process always is taking place. It also explains the aerobic reactions and the importance of the electron acceptor in biological systems.

This sort of diagram (Fig 2) permits the demonstration in the cube of a region corresponding to the methilotrophic and methanogenic microorganisms. If methilotrophs are not considered autotrophic bacteria,[11] the processes carried out by this group of microorganisms may be drawn in a vertical plane between the autotrophic and the heterotrophic phases, and in the aerobic region. The processes developed by methanogenic bacteria are also included in the vertical plane, but in the anaerobic region (Fig 2). The latter representation clearly displays the special metabolic features which characterize those microorganisms and require them to be considered separately. If methilotrophic and methanogenic bacteria were considered as autotrophs, the vertical semiplane would be developed as oblique planes into the regions I and II respectively. Otherwise, if the latter microorganisms were considered to be heterotrophs, the oblique planes should be drawn into regions IV and III respectively. The degree of description of the main reactions involved in each process will be useful for deciding the place in the cube.

We stress the versatility of the proposed diagram on including other details such as the intracellular availability of NADH or NAD^+, for reducing or oxidizing any organic compound used as energy or nutrient source (not shown).

The Nitrogen Cycle

By using the same approach, we propose the following scheme for the nitrogen cycle (see Fig 3). The regions described previously as I, II, III and IV, have the same meaning as in Fig 2. The most highly oxidized (NO_3^-) and reduced (NH_3) nitrogen compounds, are placed on opposite planes, according to their degrees of oxidation. Anaerobic respiration is described by the lower oblique plane in region III, corresponding to the anaerobic heterotrophic space. Nitrate reduction or denitrification,[5] will move the internal redox equilibrium towards NAD^+, interrupting every fermentation process. The former could be linked to the assimilative reduction of nitrate which could also be observed on the vertical plane in the anaerobic and aerobic phases.

The aerobic oxidation processes of nitrogenous compounds, carried out by chemolithotrophic bacteria, is drawn in region I of the cube (Fig 3). If the anaerobic reduction of nitrate induces an intracellular enrichment in NAD^+, the aerobic oxidation of NH_3 would induce a corresponding enrichment in NADH. That is the case of the metabolic reactions displayed by the chemolithotrophic nitrogen bacteria. This group of microorganisms also demands a high amount of NADH (NADPH) for CO_2 assimilation.[9]

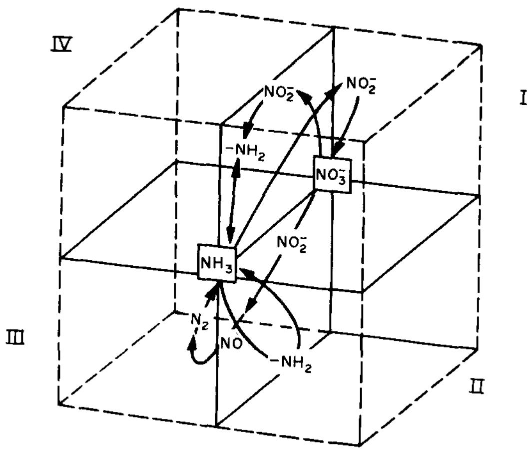

Figure 3 Three dimensional arrangement of the nitrogen cycle

The Sulfur Cycle

For the sulfur cycle, Fig 4 represents the main biological processes involved. Aerobic and anaerobic assimilative sulfate reduction can be observed in the vertical plane, between the autotrophic and heterotrophic hemispaces. Anaerobic reduction of sulfate (anaerobic respiration) is represented in region III of the cube.[4,13] The processes which represent the oxidation of reduced sulfur compounds are placed in region I (aerobic) and region II (anaerobic), and both into the autotrophic zone. The line corresponding to the anaerobic oxidation of sulfur compounds cannot be represented in Fig 3 for the nitrogenous compounds, since it has not yet been described a similar process for reduced nitrogen compounds. The latter, constitutes a great difference that emerges from the cycles and are stressed by such a three dimensional (3-D) representation. The anaerobic oxidation of sulfur compounds therefore can be considered as a physiological advantage developed by photoautotrophic bacteria, to overcome the high demand of NADH (NADPH) for supplying reducing power to the CO_2 assimilation process.[8] Other reduced sulfur intermediate compounds may be included in the semiplane which represents these sorts of processes,[7,8] depending on the complexity level we wish to show. Aerobic oxidation of sulfur compounds is described in region I, similar to that for the nitrogen cycle in Fig 3. Chemolithotrophic sulfur bacteria are involved in these processes.

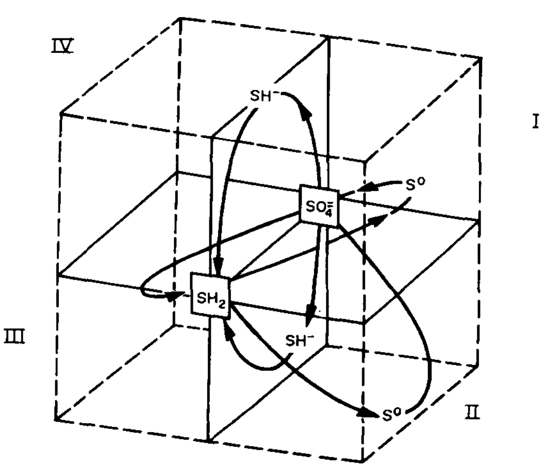

Figure 4 Three dimensional arrangement of the sulfur cycle

An Integrative Viewpoint for the Element Cycles

The possibility of assigning a direction and a sense to the biological processes involved in the carbon, nitrogen and sulfur cycles allowed us to consider a 3-D arrangement to represent the three cycles of the above elements. This is feasible, due to the 3-D distribution assigned to each of the biological processes represented in Figs 2–4. The last feature could not be accounted for with the traditional 2-D representation of the element cycles.

Fig 5 shows a simplified picture of the carbon, nitrogen and sulfur cycles all together. The space orientation of each biological process is according to Figs 2–4, with simplification in order to clarify the scheme. It offers a comprehensive integration of the most important processes which participate in the turnover of the elements in nature.

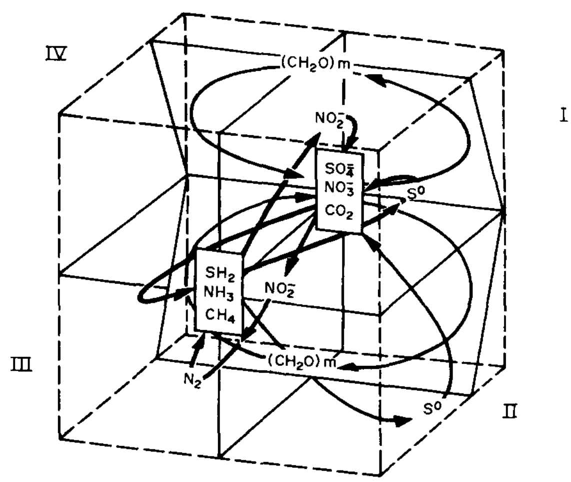

Figure 5 Three dimensional representation of the carbon, nitrogen and sulfur cycles into the four-region cube

In region I, aerobic autotrophic organisms are found. If the 'electron donor' is light, algae and plants will participate: in the dark, chemolithotrophic nitrogen and sulfur bacteria may be found.

In region II, only photoautotrophic bacteria will be found: life will only be possible if light is present.

In region III, the anaerobic heterotrophic way of life takes place. Fermentation and anaerobic respiration (nitrate and sulfate reduction), contribute to the enrichment in organic matter to those anaerobic ecosystems. In these heterotrophic habitats, life does not depend on light, although some facultative phototrophic bacteria and other higher organisms may develop.

Region IV, represents the space devoted to aerobic heterotrophic processes in which only the oxidation of organic substrates occurs. Preformed organic compounds, from anaerobic or aerobic processes, produced by autotrophic organisms, are used for providing carbon and energy to other aerobic or anaerobic obligate heterotrophic organisms.

Many other conclusions can be inferred from this 3-D representation of the element cycles (Fig 5). The conspicuous population of microorganisms which can be detected in each region does not exclude the possibility of finding some of them displaying the capacity to develop in two or three regions. When such microorganisms are detected, none of them can display processes situated in different regions at the same time. For example, if *E coli* can be placed according to its metabolic features in regions III and IV, it means that one condition must exclude the other. When *E coli* develops an aerobic metabolism, it cannot ferment or reduce nitrate simultaneously. This feature may be a useful example for introducing the students to the notion of metabolic regulation. Another interesting example is the recently reported novel energy metabolism involving fermentation of inorganic sulfur compounds.[14] Thus *Desulfovibrio sulfodismutans* can oxidize sulfide and other reduced intermediate compounds anaerobically to sulfate while a reduction to sulfide is also taken place. When this sort of metabolism is displayed, *D sulfodismutans* can use only acetate as the sole carbon source, but when the carbon source is other than acetate (lactate, ethanol, propanol, etc) *D sulfodismutans* grows only by dissimilatory sulfate reduction, producing sulfide from sulfate. In both cases the metabolism of this microorganism may occupy region III in the cube, but depending on which of them is taking place, the line representing the sulfate reduction will change to a double sense one in order to describe the anaerobic heterotrophic oxidation of sulfur compounds. The variability depends on the microorganisms, but each subspace in the cube could constitute a closed region. This point of view of the biochemical processes which take place in nature, will be very helpful on teaching environmental biochemistry.

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The Concise Encyclopedia of Biochemistry*

An account of the trials and tribulations of producing the first edition of the Concise Encyclopedia has already appeared on the pages of this journal (*Biochemical Education* (1983) **11**, 147–149). The first edition was a translation of the German publication, 'Brockhaus ABC Biochemie', supplemented and updated with some extra entries. The original, chubby little German '*Handbuch*' still sits on my shelf, but it is barely recognizable as the ancestor of the modern publication by de Gruyter; the format has changed, the type-face has changed, and the content and scope have been greatly enlarged. This transformation has occurred in two stages, ie the first, and now the second English edition. Such is the increase of material that Dr Weber of de Gruyter telephoned me at one stage to enquire whether it was still valid to use the term 'concise' in the title. Bearing in mind that my Concise Oxford Dictionary has over 2500, larger than A4 pages of small print, I assured him that this problem need never concern us. From the reader's point of view, the second edition is a greatly expanded and updated version of the first edition. For Dr Eagleson and myself, preparation of the first and second editions represented qualitatively different exercises. All the academic energy and real time orginally dedicated to the translation exercise could now be redirected to collecting and classifying new material. In this task, we were greatly assisted by colleagues and readers, who made suggestions and offered advice; not infrequently the reason for recommending an entry was "it would be useful for students". It was one of my former doctoral students, now back in Khartoum and teaching preclinical biochemistry to medical students, who suggested the entry on 'Inborn errors of metabolism'. I hope he is satisfied with the 16-page entry, as well as the new 3-page entry on 'Hemoglobinopathy'. On the other hand, the new entry on 'Recombinant DNA technology' was obviously necessary in view of the rapid progress in this field, and we were greatly assisted in its preparation by colleagues actively engaged in research in this area. There is also much new material on muscle biochemistry, and the section on vitamins has been thoroughly reorganized. On closer inspection, many entries, which appear to be unchanged from the first edition, will reveal additions and alterations. For example, the entry on 'Protein', although largely unchanged, now contains information on the latest state-of-the-art techniques for automatic sequencing. It is a measure of the pace of development of biochemistry that genetic engineering and DNA cloning received scant

*The Concise Encyclopedia of Biochemistry is published by Walter de Gruyter, Berlin, at DM148 (1988). See p 246.