Folic acid sources and medical application

Vitamins are essential nutritional compounds that contribute to a variety of functions, including metabolic maintenance and cell growth /1/. Each vitamin has particular roles that cannot be fulfilled by other vitamins in the same manner. Folic acid, often cited as a "deficient" vitamin, plays an important role of which many are not aware, although it is present in many foods, such as fruit, vegetables and whole grain products. The first scientific encounter with folic acid was in 1931 in a study by Wills, in which eating yeast was found to correct anemia during pregnancy, and Day, who found that moneys with aplastic anemia due to poor nutrition could be cured with liver extracts. In 1940, Snell et al. described an essential factor for the growth of Lactobacillus casei. Pure folic acid was isolated three years later by Stokstad and crystallized by Pfiffner. The structure of this antianemia, growth or Lactobacillus casei-factor, to mention only a few of the terms then used to refer to folic acid, was elucidated in 1946 by Angier et al. by means of degradation reactions and synthesis (reviewed by Koebnick /2/). Most of the functions performed by folic acid in the transfer of C1 units in organisms were determined by 1951 /3/. After that, little attention was paid to folic acid for a longer period of time. Recently, however, attention has been focused on it once again, because the latest research shows that it is not only a critical vitamin for nutritional maintenance, but it is crucial medical factor for embryonic development and it has a positive effect on vascular diseases, leukemia as well as on psychological health and the brain. Folic acid does not exist in a pure state naturally. It is a "pure synthetic product obtained through isolation" /4/ and is only found in foods to which folic acid has been added as a supplement, such as some breakfast cereals, salt and flour. The name "folic acid" is derived from the Latin word "folium" (leaf), because the substance was first isolated in 1941 from four tons of dried spinach leaves /5/.

Structure of folic acid

Folic acid belongs to the group of water-soluble B vitamins (formerly known as vitamin B9 or Vitamin Bc) and refers to a group of over 100 compounds with very similar properties (folates). The term "folates" refers to the folic acid derivatives occurring naturally in plant and animal nutrition, whereas the term "folic acid" refers only to pteroylglutamic acid. Folic acid stands for N-[4-(2-amino-3,4-dihydro-4-oxo-6-pteridinylmethylamino)-benzoyl]-L-glutamic acid and besteht of a 2-Amino-4-hydroxypteridine ring to which the amino group of p-aminobenzoic acid is bonded via the C6 methylene group and where the carboxyl group forms an amide bond with L-glutamic acid. The substructure formed by the pteridine ring system and p-aminobenzoic acid is referred to as pteroic acid (Figure 1).
The first building block of folic acid, the pteridine ring, is the basic skeleton of the pteridines and is found in hormones and insect dyes. The second building block of folic acid, p-aminobenzoic (PABA), is a key growth hormone for bacteria (also previously known as growth hormone H or vitamin H'). Bacteria require PABA for the synthesis of folic acid. L-glutamic acid, the final folic acid component, is an important transmitter in the central nervous system. It is also used in the food industry as a flavor enhancer, mostly in the form of monosodium glutamate (MSG).

More than half of the folic acid compounds exist as polyglutamates, i.e. up to eight glutamine components are bound to the actual folic acid molecule via the gamma-carboxyl group (pteroylpolyglutamic acids). These must first be split off during the digestive processes, whereas the monoglutamates can be more easily absorbed in the intestine as "free" folic acid. The bioavailability of naturally occurring folates is thus only about 50 to 70%, whereas supplemented synthetic folic has an availability of up to 95% and folic acid in pill form is nearly 100% /1/.

The biologically active forms of folic acid are 5,6,7,8-tetrahydrofolic acid (THF) and its derivates. There are four different coenzyme forms found in humans: 10-formyl-THF (also known as N-10-formyltetrahydrofolate, Citrovorum factor or Leucovorin); 5,10-methyl-THF; 5,10-methylene-THF and 5-methyl-THF /4/.

The role of folic acid in metabolic processes

In its active form, folic acid is involved in key metabolic processes that can be subdivided into the categories of protein metabolism and nucleic acid metabolism. Folic acid coenzymes participate in the degradation and formation of various amino acids. Thus THF is involved in the degradation of histidine as well as the conversion of serine to glycine and of homocysteine to methionine. Folic acid has an important influence on DNA synthesis and protein biosynthesis and thus a high significance for fundamental life processes /6/, so that a deficiency of folic acid is recognizable by specific dysfunctions.

The tetrahydrofolic acid 5,6,7,8-THF is the biologically active form of folic acid. Figure 2 shows its formation from folic acid.
THF is created by the reduction of folic acid via the dihydrofolic acid (DHF) intermediate catalyzed by the two NADPH/H⁺ dependent enzymes, folate reductase and dihydrofolate reductase. THF and its derivatives constitute the coenzymes for transmission of C1 units such as methyl, formyl, formate or hydroxymethyl functional groups. The carriers of these C1-units in the "one-carbon metabolism" are always the nitrogen atoms at the fifth or tenth position of the pteroyl group. In general, the transfer reactions between the donor and acceptor take place without a change in oxidation state. The transfer can be divided into three stages:

a) C1-bonding to THF
b) Activation of the complex via isomerization
C) Transmission of the C1-unit to the acceptor molecule.

**Folic acid – from medical use to waste water technology**

In the biocoenosis of sewage treatment plants as well, there is too little of the vitamin folic acid, due to its limited stability in aqueous solution and it therefore has a limiting effect on the metabolic steps in the process. The product DOSFOLAT®XS, which can be added to the treatment plant, contains folic acid in stabilized form as its active ingredient. Here, the folic acid serves the particular function of regulating the 1-carbon metabolism, i.e. the breakdown of formic acid with oxygen to carbon dioxide and water. Not only does DOSFOLAT®XS contain sufficient folic acid, but additional biochemical cofactors as well which act synergistically to effect a fast conversion of metabolic activity in the activated sludge process. Without folic acid, the metabolic processes in the cell membrane would occur at a significantly lower rate despite sufficient supply of air. Without folic acid, key parts of the biocoenosis work with a multi-step, slow "primitive metabolism" - such as that which is prevalent for example in the deep sea, where there is also no folic acid. No habituation has been observed in the process.
even after years of DOSFOLAT® use. DOSFOLAT®XS is available as a concentrate that is easy to administer and has an effective delivered and has the effective power per kilogram of about 25 to 30 kg of unstabilized folic acid.

Systematic evaluation of operations data with the use of DOSFOLAT®XS in more than 60 municipal and industrial sewage treatment plants North and South America from 1995 to 2003 provided the basis for reliable deployment criteria for DOSFOLAT®XS for various biological sewage treatment facilities. Here it could be proven that the influence of DOSFOLAT®XS in the activated sludge process significantly reduced the production of excess sludge by about 50% /8/. In the meantime there are about a dozen sewage treatment plants in Germany where folic acid is being used to reduce excess sludge and increase process stability. This will be discussed in the report to follow.

**Dosing station:**
Dosing of the stabilized folic acid takes place in the return sludge stream (Figure 3). Care should be taken that there is sufficient turbulence at the dosing station to ensure thorough mixing in of the folic acid. The precipitant dosing station (P elimination) must not be adjacent to the folic acid dosing station.

![Dosing system for stabilized folic acid](image)

**Figure 3:** Dosing system for stabilized folic acid (sewage treatment plant in Bavaria an der Isar)
Dosing rate:
The dosing rate of the stabilized folic acid is designed for moderate dry weather runoff and is to be added continuously throughout the 24 hour day. The folic acid dosing rate required for example for a dry weather runoff of 5,000 m$^3$/d is determined as follows:

- First 15 days at 0.5 ppm ("shock dosing"),
  i.e. 5,000 m$^3$ waste water/day · 0.5 ppm yields 2.5 liters per day of DOSFOLAT®XS (concentrate), or at a dilution of 1:400 the daily dosage of DOSFOLAT®XS solution would be 1000 liters (required max. pumping rate: 1000/24 = 41.7 liters/hr).

- After that, an operating dosage of 0.1 ppm is applied,
  i.e. 5,000 m$^3$ waste water/day · 0.1 ppm yields 0.5 liters per day of DOSFOLAT®XS (concentrate), or, at a dilution of 1:400 the daily dosage of DOSFOLAT®XS solution would be 200 liters (required max. pumping rate: 200/24 = 8.3 liters/hr).

Excess sludge discharge procedure

- First 15 days at 0.5 ppm ("shock dosing"):
  After beginning dosage of the folic acid, the discharge routine for sludge, i.e. the daily removal of excess sludge (tons of solid material per day), is to be kept the same as prior to the folic acid addition. During this period, the folic acid slowly takes effect and the biocoenosis achieves a higher intensity in the C1-metabolic process. The self-adjusting biocoenosis is documented by microscopic images. The acceleration of the 1-carbon metabolic process during this period decreases the daily growth of sludge and thus increases the age of the sludge. Consequently, no increase of the solids content in the activated sludge tank is to be expected at the same BSB$_3$ load in the feed to the sludge tank.

- After that, an operating dosage of 0.1 ppm is applied:
  After the 15-day "breaking-in phase", the discharge of excess sludge is reduced in stages – depending on the processing technology of the sewage treatment plant, by about 10% per week for example. The daily sludge production will continue to decline, accompanied by a further increase in the age of the sludge. The solid material content in the activation tank can also be, analogous to experience with other operating materials such as tensides, kept nearly constant (variations of the solid material content in the tank however can always be observed as a consequence of rainfall events in mixed systems or changes in the supply loads). The reduction of excess sludge discharge is to be continued in stages until an equilibrium state has established itself between the discharge and the daily production of excess sludge in the activation tank. This state of equilibrium has been reached if further reduction of the excess sludge discharge causes the solid content in the activation tank to start increasing again; thus at this point less excess sludge is removed than is actually formed in the activation tank. Then it may be necessary to increase the removal by a few percent in order to reach the equilibrium state again. Im subsequent Zeitraum wird dann der Überschusschlammabzug – wie er sich im equilibrium state eingestellt hat – unverändert beibehalten. A "readjustment" of the excess sludge in the subsequent period of operation is only necessary if the load situation at the activation stage changes.

Documenting the effects of treatment

Mass balancing has proven to be a useful tool for determining the biologically reduced incidence of excess sludge, regardless of whether the effect of the DOSFOLAT®XS documented in the before/after comparison is
- the specific biological excess sludge production or
- the absolute biological excess sludge masses

Which procedure is applicable depends on the operating data available and the load situation of the biological stage (individual case observation) in the before and after comparison. Documentation of the effect via the absolute biological excess sludge masses can be done if the supply loads and the solids content in the activation tank (equal sludge load) are nearly the same both in the reference as well as the experimental period. The results of both procedures lead to a conclusion regarding the reduced biological excess sludge [t TS] during the period of observation.
Results at selected sewage treatment plants

Table 1 summarizes the experiences at the treatment plants in Germany currently operating with DOSFOLAT®XS.

Table 1: Sewage treatment plants using DOSFOLAT®XS (as of May 6, 2004)

<table>
<thead>
<tr>
<th>Treatment plant</th>
<th>Med. dry weather runoff</th>
<th>Med. COD concentration</th>
<th>Start DOSFOLAT dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrial Sewage treatment Chemical industry (NRW)</td>
<td>1,200 [m³/d]</td>
<td>1,200 [mg/l]</td>
<td>17 Oct 2003</td>
</tr>
<tr>
<td>STW municipal (Saxony-Anhalt)</td>
<td>450 [m³/d]</td>
<td>1,500 [mg/l]</td>
<td>08 Dec 2003</td>
</tr>
<tr>
<td>Biological municipal (Lower Saxony)</td>
<td>2,300 [m³/d]</td>
<td>450 [mg/l]</td>
<td>26 Mar 2004</td>
</tr>
<tr>
<td>municipal (Bavaria)</td>
<td>6,800 [m³/d]</td>
<td>365 [mg/l]</td>
<td>31 Mar 2004</td>
</tr>
<tr>
<td>Papermill (Schleswig-Holstein)</td>
<td>20,000 [m³/d]</td>
<td>1,400 [mg/l]</td>
<td>10 Apr 2004</td>
</tr>
<tr>
<td>Bavaria municipal (Bavaria)</td>
<td>382 [m³/d]</td>
<td>1,096 [mg/l]</td>
<td>19 Apr 2004</td>
</tr>
<tr>
<td>Zweckverband Abwasser beseitigung Mittlerer Itzgrun (Bavaria)</td>
<td>3,000 [m³/d]</td>
<td>589 [mg/l]</td>
<td>30 Apr 2004</td>
</tr>
</tbody>
</table>

At three treatment facilities, the modification of the sludge discharge process is already concluded, so the first operational results can already be presented. The plants in question are

- die industrial sewage treatment plant ISP at (Figure 4),
- the middle east German MEG(Figure 5), and
- The lowerSaxony Plant LSP(Figure 6).

As previously explained, after the introductory phase for the stabilized folic acid, the discharge of excess sludge is reduced stepwise each time by 10% at each sewage plant at predetermined intervals. The magnitude of these intervals based on previous experience is between 3 and 7 days and is presumably also dependent on the age of the sludge in the sludge activation system in the period prior to dosing with the stabilized folic acid. It should be noted that the size of the daily sludge discharge as "external intervention" in the system by operating personnel need not always correspond to the daily production of excess sludge. With an equal load situation or conditions of the biocoenosis, a deviation of the daily sludge discharge from the actual daily
incidence of excess sludge can thus lead to a decrease or increase in the solids content of the activation tank, depending on whether more is removed than is produced each day or less. A decrease or increase of the solids content can thus occur even with a change in the sludge discharge process after the introductory phase (15 days) of stabilized folic acid addition if the 10% reduction of the discharge at the prescribed interval does not reflect the actual incidence of excess sludge. The load data for the activation tank system (for example COD load) as well as the history of the solids content in the activation tank, are thus to be taken into account.

The effect of the stabilized folic acid on the daily production of excess sludge can be seen in Figures 4 through 6.

![Figure 4: ISP sewage treatment plant - TOC load as activation load magnitude, reduction of the excess sludge discharge as well as the equilibrium solids content in the activation tank](image)

At the ISP industrial sewage treatment plant, due to alkylphenolic residues and higher COD concentration (average 1200 mg COD/l), the ongoing operating dosage of stabilized folic acid after the initial phase was set at 0.2 ppm (usually this is done only with levels above 2,000 mg COD/l, below that 0.1 ppm is typically used). During the conversion of the sewage discharge regime of the ISP sewage treatment plant (Figure 4), a decline in the solids content was initially observed in the activation process. Later in the process, with increasing reduction of the excess sewer discharge, the initial solids content is restored. The mean TOC load (TOC load) in the observed period did not change and is within the usual fluctuation range for sewage treatment plants. At the end of April the excess sewage discharge increased slightly, as the solids content increased. At this point, more sewage was discharged than was produced on a daily basis. The time history of the operating parameters indicates that a “cautious approach” to the newly adjusted equilibrium achieved by the stabilized folic acid between the excess sewage discharge and the increase in excess sewage is required, in order to draw off only the excess sewage which is actually produced during a certain period.
The excess sewage discharge was reduced at the ISP sewage treatment plant by a total of 67% compared to the reference period without stabilized folic acid. Due to the fluctuation in solids content, in order to evaluate the reduction of the actual sewage produced compared to the reference period, a weight assessment which included the activation and the treated sewage disposed of was necessary. This weight assessment indicated the reduction of excess sewage by 30%.

In the plant trials with stabilized folic acid at the MEG and LSP sewage treatment plants, a significant reduction of the excess sewage was also established (Figures 5 and 6). These trials demonstrated that the solids content during the activation process in the modification of the sewage discharge regime (reduction of the excess sewage discharge) and during the following period remained constant for the most part, despite slightly increasing COD loads in the feed to the biological process. The dimension of the daily sewage discharge as an “external intervention” in the system is thus roughly identical with the daily production of excess sewage for both systems. The solids content of the discharged excess sewage varied only slightly in the periods under review, so that the reduced excess sewage discharge was approximately equal to the targeted reduction in excess sewage (50% and 60% respectively) for both systems. The accompanying mass balances which were carried out also confirmed these results.

Figure 5: The MEG sewage treatment plant - COD load as activation load magnitude, reduction of the excess sludge discharge as well as the equilibrium solids content in the activation tank.
Figure 6: The treatment plant LSP - COD load as activation load magnitude, reduction of the excess sludge discharge as well as the equilibrium solids content in the activation tank

In contrast to the ISP treatment plant, the age of the sludge in the MEG and LSP treatment plants (both with aerobic sludge stabilization) prior to dosing with stabilized folic acid was above 30 to 40 days, whereas at Sasol it was significantly less than 20 days. At the ISP plant variations of the solids content in the activation tank were documented during the investigations. Similar results have been observed in the tests currently in progress at the sewage treatment plant in Bavaria (the sludge age was also less than 20 days here before the addition of stabilized folic acid). At the MEG and LSP facilities, however, no significant change in the solids content was observed. Whether this should lead to the conclusion that plants whose sludge is less than 20 days old will experience periodic changes in the solids content (at otherwise equal loads) during the establishment of a new equilibrium state when compared with plants having older sludge, and whether these plants should have the time interval for 10% reductions of the excess sludge discharge extended to about 7 days instead of the 3 to 5 for plants with older sludge needs to be clarified through additional operational testing.

As part of operational testing with stabilized folic acid, changes in the biocoenosis were documented once a week by microscopic studies. It was found that the higher microorganisms developed somewhat more slowly given the same feed of active biomass and the acceleration of metabolic processes in the sludge activation system, despite the fact that the age of the sludge was doubled more or less as a result of reductions in the discharge of excess sludge.
Summary and future prospects

Due to its instability in aqueous solution, the vitamin folic acid is not present at high enough levels in the biocoenosis of treatment plants and therefore has a limiting effect on the metabolic processes of sludge activation. Folic acid is particularly important in regulating the 1-carbon metabolism of the microorganisms and leads to an increase in metabolic activity.

DOSFOLAT®XS, which contains stabilized folic acid as its active ingredient, allows the external addition of folic acid to sewage treatment processes. The continuous dosing rate of stabilized folic acid for moderate dry weather runoff triggered over a 24 hour period is designed to be 0.5 ppm initially ("shock dosing") and thereafter the operating dosage is 0.1 ppm. According to the information currently available, evaluations of initial operation testing with this product in Germany indicate that a reduction in excess sludge on the order of 45 ± 15% is possible. No habituation has been observed in the process even after years of DOSFOLAT® use. DOSFOLAT®XS is available as a concentrate that is easy to administer and has an effective delivered and has the effective power per kilogram of about 25 to 30 kg of unstabilized folic acid.

Further investigations, for example for evaluation of the effects of stabilized folic acid on the reduction of the residual load in separated sludge water from sludge dehydration, increase of the process stability as well as release of additional tank capacity (reaction volumes) in facilities whose capacity is at full use or overloaded will be the object of future research projects.

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