# A brewer's biochemistry

By **Professor Charles Bamforth**
Department of Food Science and Technology, University of California, Davis, USA.

## Part 5: Metabolism

**This is the fifth and final article of the series aiming to position malting and brewing in biochemical terms for the benefit of those who have received no training in this area of science. Readers lacking a formal scientific training will find basic chemical principles described in the first article of the series.**

So far in this series we have discussed the various molecules, pretty big ones at that, from which all living cells are composed. In this final article I will discuss the way in which these substances are inter-converted – that is built up and broken down in a series of reactions that is known as *metabolism* and which comprise the very essence of life.

The basis of metabolism is the same in all living cells, whether they are from barley, hops, yeast or brewer. There is a series of reactions, generally referred to as *catabolism*, in which a feedstock is broken down to generate energy. A further series of reactions, termed *anabolism*, is responsible for building up the cellular components from which the cell is made and which are necessary for the jobs performed by the cell. This is illustrated in Figure 1 for a cell of brewing yeast.

Energy is a key word in the context of metabolism. For the cells that we are primarily concerned with in the brewing industry, the main demand for energy is to fuel the biosynthetic (anabolic) reactions and to transport foodstuffs into the cells and waste products out of the cells (Figure 2).

### The energy currency

Living cells use a molecule called ATP as energy currency. (We needn't worry about what the initials stand for, but suffice to say it is not the Association of Tennis Professionals). In catabolic reactions that release enough energy, it is trapped in the form of ATP. The way the cell does this is to take a molecule called ADP and stick some phosphate onto it (Figure 3). The attachment (bond) formed is a high energy one.

When the cell needs some energy it turns to its ATP and reverses the reaction. The phosphate is split off, ADP is re-formed, and the energy is made available.

### Energy-generating reactions

Want to get warm? Then we burn wood or coal or some such. Want to breathe life into the fireplace? Then we pump in some oxygen – once upon a time with bellows. The main ingredient in, say, wood is carbohydrate. So, taking the above at face value, if we react carbohydrate with oxygen we release energy. The smoky gas you see is primarily composed of water vapour and carbon dioxide.

And so we have the famous reaction:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6\,H_2O + \text{energy}$$

Or, in words:
Carbohydrate + oxygen → carbon dioxide + water + energy

This is exactly the same reaction that occurs in most cells that burn up sugar when they are living in the presence of oxygen. It is the principle energy generating process in a germinating embryo and in you and me.

In fact the reaction doesn't happen all in one go, with an almighty release of energy, somehow captured as ATP. Rather it happens gradually and in an extremely organised way through a long sequence of reactions, each of them catalysed by a different enzyme (see the first article in the series for information on enzymes). The process is called *respiration*.

First of all the cell needs to get the sugar into the cell. Let's take brewing yeast as our example here. It makes several proteins that have the role of latching on to the various sugars and transporting them across the cell membrane. Energy is expended in achieving this.

What happens next comes as something strange to the non-biochemist: the cell takes some ATP, rips of the third phosphate and sticks it on to the sugar. (Let's stick with glucose as our sugar – more complicated carbohydrates tend to be converted into that as the starting point of metabolism.)

And so we have glucose 6-phosphate, or G-6-P (the phosphate is attached to carbon atom number 6 of the glucose – see the second article). G-6-P is an "activated" form of glucose. It is primed for action. Just think of glucose as one of those little cars they give you at Macdonald's, the sort you pull back to brace the spring. Adding the phosphate to glucose is tantamount to rolling back that little car: when you let go it zooms into action.

There is no real point for our purposes in discussing the individual reactions (there are more than a dozen of them) that are involved in breaking down the G-6-P. We can restrict ourselves to some generalities (Figure 4):

- the G-6-P is changed (isomerised) into a related molecule called F-6-P (F stands for fructose), which is cranked up to yet a higher energy level by the addition of another phosphate from ATP (makes F-1,6-diP)
- the F-1,6-diP, which contains 6 carbon atoms in the sugar portion (see my second article) is split to produce 2 molecules of pyruvic acid, which contains 3 carbon atoms.
- In so doing, the cell has oxidised the sugar.
- There are two stages in the pathway where the phosphates added on to the sugar are released and are recaptured as ATP. As two molecules of pyruvate are produced, then we double this value up. In other words, we have expended two ATPs in activating the sugar and have produced a total of four. Net effect: capture of energy as two ATPs.

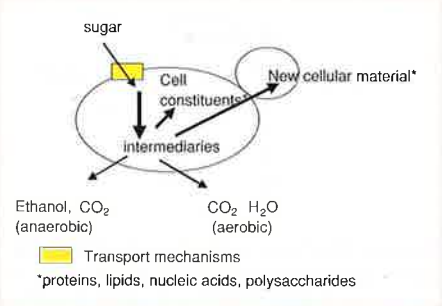


*Figure 1. The essence of metabolism in yeast.*

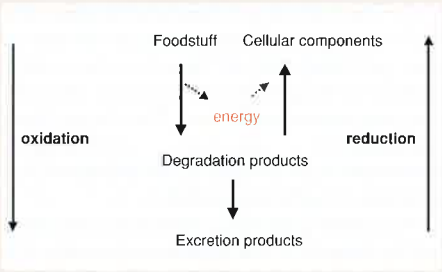


*Figure 2. Catabolism largely oxidative and energy generating, producing intermediates that are either taken via reduction and energy consumption to the level of cellular constituents (the polymers I have described in this series) or are converted into excretion products (such as ethanol and carbon dioxide).*

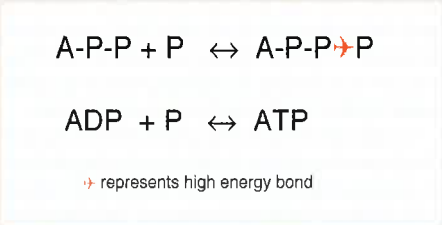


*Figure 3. The capturing of energy as a bond in ATP. In the reaction headed towards the right energy is captured (catabolic reactions). When the reaction moves to the left energy is released and utilised (anabolic reactions).*

### Oxidation

I described oxidation and its opposite (reduction) in the chemistry crib in my first article in the series. For our purposes here I am going to refer to oxidation as a removal of hydrogen from a molecule, whereas reduction is the addition of hydrogen.

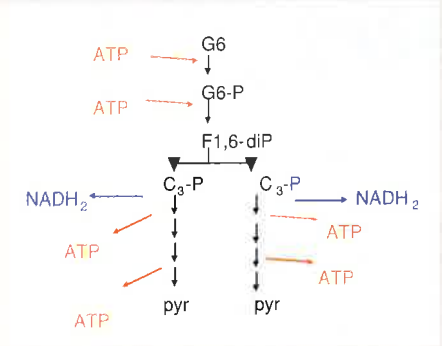


*Figure 4. The essence of glycolysis. $C_3P$ are 3-carbon sugars energised with phosphate; pyr = pyruvic acid.*

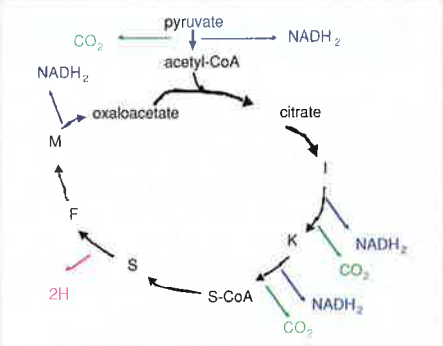


*Figure 5. The essence of the Krebs cycle.*

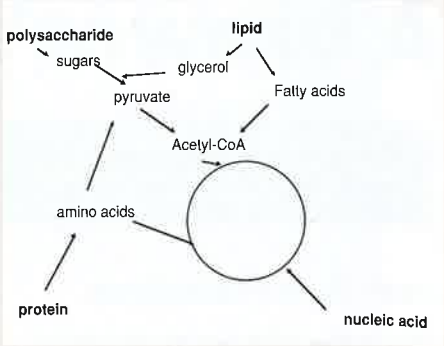


*Figure 6. Catabolic routes into the backbone of intermediary metabolism.*

So when glucose is oxidised to pyruvate, there is a removal of four hydrogens. Where do they go? Well, just as there is a "universal" energy currency (ATP) so there are a couple of universal hydrogen carriers. The first is called nicotinamide adenine dinucleotide, henceforth to be referred to as NAD (for which you will no doubt be grateful).

This tends to be the molecule that receives the hydrogens in the oxidative reactions. For sake of convenience we will say that $NADH_2$ is produced. The second hydrogen carrier is NADP (it has an extra phosphate on it). Its role tends to be to donate hydrogen to reactions (mostly anabolic) that need hydrogen – so here we are talking about $NADPH_2$ going to NADP.

Thus, when glucose is converted to pyruvate, two molecules of $NADH_2$ are produced. If the cell merrily continued chewing glucose then pretty soon all of the NAD would be soaked up as $NADH_2$ and no more NAD would be available. Everything would grind to a halt. So the NAD needs to be regenerated.

In cells growing in the presence of oxygen this is achieved by transferring the hydrogen to oxygen through a series of carriers called cytochromes. Without going into details about precisely how it is done (although you may be interested to know it was worked out by a bloke in a big house near Bodmin Road in Cornwall), in this transfer a total of three molecules of ATP can be made for every molecule of $NADH_2$. For this is the stage at which the energy of oxidation is released and captured in a controlled way. Two molecules of $NADH_2$ need to be oxidised, so that is a total of six ATP's at this stage.

What if the cell is growing without oxygen, though? How then does it return its $NADH_2$ to NAD? The answer is by a *fermentation* route. The $NADH_2$ is used to reduce some molecule other than oxygen. This varies between organisms, but let's concern ourselves only with *Saccharomyces cerevisiae*.

What yeast does is to strip out a carbon dioxide molecule from the pyruvic acid that it has produced. This *decarboxylation* produces acetaldehyde. It is this latter molecule that receives the hydrogens from $NADH_2$ in a reaction catalysed by alcohol dehydrogenase. The product, of course, is ethanol.

$$\text{pyruvate} \rightarrow \text{acetaldehyde} + CO_2$$
$$\text{acetaldehyde} + NADH_2 \rightarrow \text{ethanol} + NAD$$

Of course when we break down glucose we produce two pyruvates and two molecules of $NADH_2$. By decarboxylating and reducing both pyruvates we can regenerate the two NAD's used earlier in the pathway of glucose breakdown (which is known as *glycolysis*).

The net effect then is

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

This is the equation of alcoholic fermentation by yeast – the very heart of brewery fermentations.

### What else can happen to the pyruvate?

This alcoholic fermentation does not occur when yeast is growing in the presence of high concentrations of oxygen. Under these conditions we have total conversion to carbon dioxide and water, according to the very first equation I put into this article. How is this achieved?

The answer is: by a pathway originally worked out by the legendary Sir Hans Krebs (see box). The pathway is a cycle, sometimes called the Krebs cycle, sometimes the tricarboxylic acid cycle and sometimes the citric acid cycle.

The jumping off point demands that the pyruvate be first oxidised to acetic acid. This involves a specific enzyme (as do all the various reactions I have and will describe). In fact free acetic acid is not produced, rather acetic acid attached to something called Coenzyme A. In just the same way that a sugar is "activated" by adding phosphate to it, so is acetic acid activated by adding it to Coenzyme A, to form acetyl-CoA.

The bare bones (shape, not detail) of the Krebs cycle is shown in Figure 5. I have deliberately not included all the names of the intermediates, but I have tried to indicate where the hydrogen and carbon dioxide emerges.

Remembering that for each glucose there are two pyruvates, then you can add up for yourself the emergence of six carbon dioxide molecules. And remember that every 2 hydrogens captured (as $NADH_2$) are oxidised to water with the release of 3 ATPs in the manner described earlier.

Quite clearly this complete conversion of

## A scientist's genealogy

ONE of my hobbies is genealogy. Thus I have traced *inter alia* my genes back to a Yorkshire weaver in the late 18th century (not necessarily welcome news for a Lancastrian) and also discovered a sister I never knew existed.

We scientists, however, have another type of genealogy. Our "father" is the guy who supervised our Ph.D. Thus my scientific old fella is Peter Large at Hull, brilliant scientist, beer *bon viveur* and loveable eccentric (see www.hull.ac.uk/php/abspjl).

In turn he did his Ph.D with Rod Quayle FRS (with whom I spent two exquisite years as a post-doc in Sheffield). This makes Rod my scientific grandfather. Smashing man.

It's when we go back a generation from that when things get a little more complex. Hard as it is to imagine these days, but Rod Quayle earned *two* Ph.D.'s, one from Bangor and the second from Cambridge. The latter was under the supervision of Lord Todd (Sir Alexander Todd), who gained the Nobel Prize for his work on the structure of the building blocks of nucleic acids. And then Rod headed off to do research with Melvin Calvin, "father" of photosynthesis who worked at our sister campus at Berkeley, and another to gain a Nobel Prize. Rod then returned to Oxford, which is where Peter Large came into his team. At that time Rod Quayle's boss was Sir Hans Krebs – yes, discoverer of the Krebs Cycle and yet another Nobel Prize winner.

So I am claiming one Nobel prize winner (Todd) as my great grandfather with two others (Krebs and Calvin) as great, great uncles!

I wonder if they might one day give out Nobel Prizes for work on bubbles in beer?

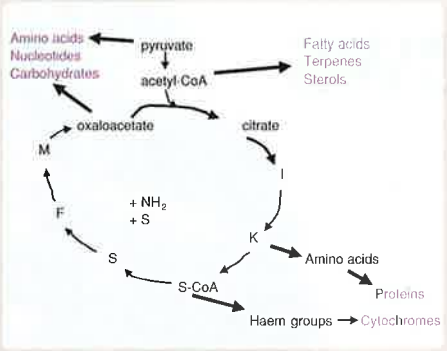


*Figure 7. Anabolic routes away from the backbone of intermediary metabolism.*

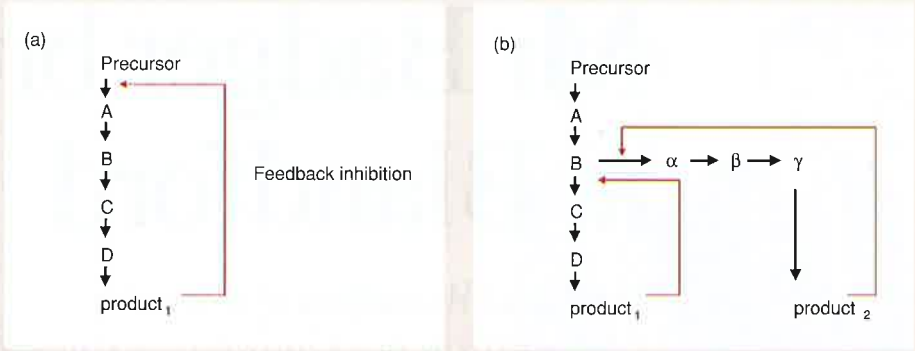


*Figure 8. Feedback control of metabolic pathways. Each of the arrows indicates a separate enzyme-catalysed reaction. In (a) unnecessary accumulation of the product can feedback to inhibit the enzyme catalysing the formation of the first intermediate (A). This avoids the wasteful accumulation of the end product but also of all the intermediates in the pathway. It is likely that the precursor itself will feedback to an earlier enzyme still, and so on, in cascades designed to ensure that only necessary molecules are produced. In (b) if the same product fed back to block the same enzyme there would be a problem, because this would prevent the formation of B, which is needed to go through the pathway to make product$_2$. Thus in this scenario, product$_1$ and product$_2$ feedback to inhibit the first enzymes **unique** to their production.*

glucose to carbon dioxide and water enables much more ATP to be produced than is captured in the anaerobic pathway. Curiously, though, if brewing yeast is confronted with a very high sugar content, it has control mechanisms (see later) which turn off the Krebs route and channel the breakdown through fermentation. This is the Crabtree effect, named after a scientist of that name. It seems that when confronted with all that potential energy, the cell wishes to avoid ATP overload.

## What about other foodstuffs?

The short answer to this question is that a cell shifts the various molecules it has available for degradation to an intermediate somewhere in this central pathway that I have described (Figure 6). If it is an endogenous store of polysaccharide (starch or glycogen, for instance) then it has enzymes that will chop the store down to glucose. Fats are converted to glycerol, which enters the pathway just above pyruvate, and acetyl-CoA. And so on.

## Making molecules

I have concentrated so far on how a cell such as yeast (but similar things are going on in a barley embryo or a you or a me) degrades molecules to produce energy.

Some of that energy is used to make the molecules that the cell needs – its proteins (including the enzymes and structural proteins), the lipids for the membranes, the nucleic acids and the storage polysaccharides, all of which I have talked about in detail in previous articles.

Although a cell can assimilate *some* materials pre-formed (for example, yeast will plug some lipids from the medium into its membranes), by and large it makes them from scratch. The molecules are built up from smaller molecules.

I needn't go into the detail of the step by step way in which specific enzyme piece together these big molecules. Rather I would simply draw attention to the fact that the building bricks are intermediates in the central pathways that I have already described (Figure 7). It is here that the other nutrients needed by a cell come into play, e.g. sources of nitrogen and sulphur.

In other words there are various fates for these intermediates.

Let's illustrate the point by considering just one such intermediate, oxaloacetate. On the one hand it is needed to capture acetyl-CoA to make citric acid to allow the Krebs pathway to cycle. However, it is also the kick off point for making a key amino acid (aspartate) which is made by sticking an amino group on to the oxaloacetate. In turn this aspartate heads off in pathways to make more amino acids (methionine, threonine, isoleucine, and lysine) and also some of the building blocks for DNA and RNA.

Separately oxaloacetate is also the start point for the synthesis of carbohydrates within the cell (gluconeogenesis), and a whole bunch of other amino acids. Some of these reactions demand reducing power, which as we have seen is delivered in the form of $NADPH_2$.

Imagine if you will the Krebs cycle as a relay race, with each of the intermediates being runners handing on the baton in sequence. Oxaloacetate is the last of the runners, but in a position to pick up a fresh baton (acetyl-CoA) ready to start the next circuit. But if oxaloacetate has got side-tracked and is legging off in other directions, it needs to be replaced from another source in order that the race can continue.

And so there are other pathways (*anaplerotic*) that top up the runner availability and allow the cycle to continue. They are schemes (too detailed to cover here) which effectively produce oxaloacetate from sources other than the Krebs cycle.

## Exerting control

Quite evidently we have a somewhat complex scenario. A multitude of interconversions is at play – it has been estimated that the very simplest of bacteria need over one thousand separate reactions. This vast metabolic "orchestra" needs to be conducted under strict control. This is achieved by a cell in various ways, but I will refer to just a couple.

First, it produces only the materials that it needs. In effect this means that it develops only the enzymes and other workhorse molecules (e.g. transport mechanisms) that are needed for it to do the jobs demanded of it. To do otherwise would be a waste.

Thus, if a yeast is growing on a high level of glucose it does not make the high levels of cytochromes needed in respiration, does not make the transporters needed for other sugars present in smaller quantities, and does not turn on the enzyme needed to convert pyruvic acid into acetyl-CoA. It will, however, switch on high levels of the enzymes that convert pyruvate into acetaldehyde and then ethanol.

When I talked about nucleic acids in the last article I discussed gene transcription and translation – the cell controls protein synthesis at this level and responds to triggers and hints from its environment in order to switch on or switch off the expression of various genes.

The second level of control is at the enzyme level itself and involves inhibition (which I talked about in the first article of the series). This is illustrated in Figure 8. Imagine that the end product of this pathway is accumulating to excess. It does not make sense to make any more.

Then it often happens that the material "feeds back" to inhibit the first enzyme unique to the synthesis of this material. It cannot feed back too far, or it would lead to the cessation of production of something that *is* needed.

## Concluding words

As a young student of biochemistry over 30 years ago I would sit in the pub, writing out the Krebs cycle on peeled-back beer mats. Sad, eh? But what beautiful symmetry: the very visualisation of what can happen in the yeast that had produced the ale before me.

I hope you have enjoyed the series – for personal tuition contact me at:
cwbamforth@ucdavis.edu.
You provide the beer mats. ■