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Purple urine bag syndrome: regulation of pyrimidine enzymes

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Dedication

To my parents, Curtis Broadway and Sharon Broadway, I am extremely grateful for everything that you do for me. You love me no matter what and support me in all that I do. To my sister, Katelyn Broadway, for all of your love and support. No matter what you are always there and I have loved watching you grow into an amazing young woman. Thank you all for always praying for me and giving me advice when I need it. Your words of encouragement are what have pushed me to achieve all that I have and all that I will.

"Therefore I say unto you, What things soever ye desire, when ye pray, believe that ye receive them, and ye shall have them."

-Mark 11:24 KJV

Abstract

Purple Urine Bag Syndrome, PUBS, is a unique condition that shows an interesting discoloration of the urine from women who have a chronic catheter. This study focused on the regulation of enzyme activities to determine the effects that may create an environment where PUBS is seen in patients with catheters. In this study, the reductive pyrimidine pathway was used to read out the bacteria *Pseudomonas aeruginosa* ATCC 15692. This microorganism expresses a pathway that synthesizes indoxyl from tryptophan. The bacteria strains were grown using various carbon and nitrogen sources. Concentrations of acetonitrile at 2 mM and 10 mM, concentrations of tryptophan at 0.2 mM and 1 mM, and concentrations of ammonia at 0.2 mM and 1 mM were added to the bacterial growth media and assay mixtures. Dihydropyrimidine dehydrogenase was assayed and found that in Glucose Uracil minimal media, adding higher concentrations of acetonitrile resulted in a 2-fold decrease in specific activity, adding higher concentrations of tryptophan resulted in a 1-fold increase in specific activity, and adding higher concentrations of ammonia resulted in a 9-fold increase in specific activity. The study demonstrates that the enzyme could be regulated by acetonitrile, tryptophan and ammonia thus indicating a means to understand the urine discoloration.

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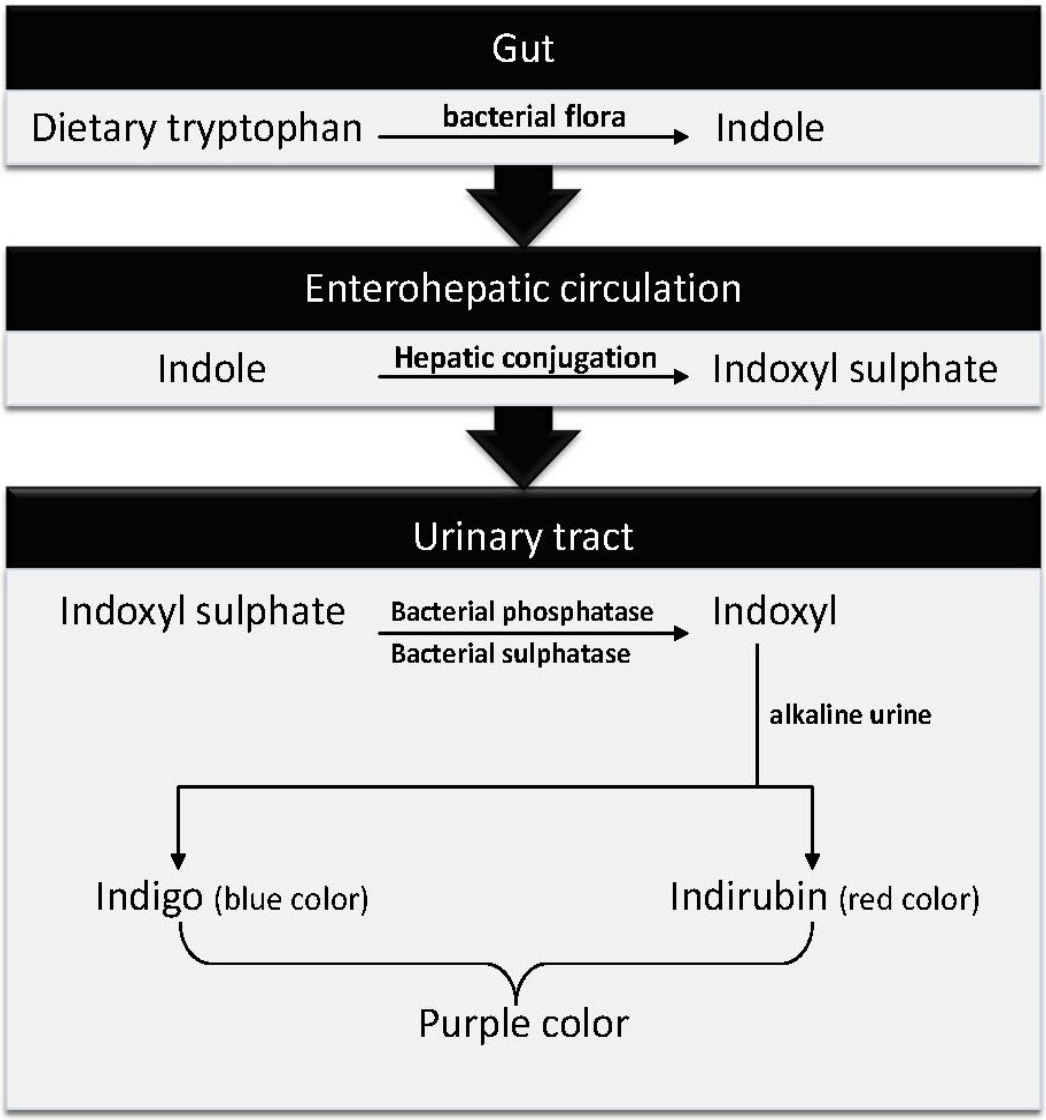
Introduction

Purple Urine Bag Syndrome (PUBS) is a condition that shows a unique discoloration of the urine (Chong et al., 2009). It is mainly found in elderly women who have a chronic catheter, chronic constipation and a urinary tract infection. In this study, the enzyme Dihydropyrimidine Dehydrogenase in the pyrimidine metabolic pathway was analyzed to examine the effects of the presence of *Pseudomonas aeruginosa* ATCC 15692 in this disorder. It was investigated if the urinary tract infection was due to an increase of *P. aeruginosa*, which, combined with nitriles, tryptophan, and a urea induced environment, by the addition of ammonia, prompted the color change.

Pseudomonas are gram-negative bacilli, which are found in soils as well as aquatic habitats. Several are plant and animal pathogens. *Pseudomonas syringae* and *P. cichrii* are known plant pathogens and *P. aeruginosa* is an opportunistic human pathogen associated with infections of the urinary, respiratory and gastrointestinal tracts (Todar, 2004; Ramphal et al., 1980). *Pseudomonas aeruginosa* was used as the microorganism in this study, for numerous reasons. First of all, it is a human pathogen that is known to give blue/green coloration in its growth media (Stratton, 1983). Also, in a case of Purple Urine Bag Syndrome, urine cultures yielded more than 100,000 colony forming units of *Pseudomonas aeruginosa, Morganella morganii,* and *Proteus vulgaris* (Chen, 2012). Lastly, it can produce the bacterial enzymes indoxyl sulphatase and indoxyl phosphatase, which are needed to combine with indoxyl sulphate to be metabolized into indigo and indirubin (purple color).

Acetonitrile, a type of nitrile that is an organic compound with a carbon-nitrogen triple bond functional group, was added to mimic catheterization in patients. The most common catheter is a nitrile catheter. Tryptophan can be found in the person's diet. These patients are most likely on a tryptophan diet, because this diet is a natural way of warding off depression. By means of serotonin production, thereby regulating sleep patterns, appetite and moods of an individual. Tryptophan is metabolized by the gut to indole, which is absorbed by the portal circulation and converted in the liver to indoxyl sulphate (Ben-Chetrit & Munter, 2012). This molecule is then excreted in the urine and, in the presence of an alkaline environment (urinary tract infection) and bacterial enzymes (indoxyl sulphatase and indoxyl phosphatase), it is metabolized into indigo (blue) and indirubin (red), which gives urine a purple color, see Figure 1 below (D'Souza & Hirzallah, 2010). Ammonia is in human urine, and the goal is to simulate, in the laboratory, the condition in the body; therefore, was added to see what changes this environment would show. Due to ammonia being a feedback inhibitor, if the results do not reveal enzyme regulation, then that would demonstrate it is acting as a negative effector (Berg et al., 2008). However, if the results show a change in enzyme activity, then that supports that the tryptophan is affecting the ammonia metabolism.

Figure 1. Metabolic Pathway of Purple Urine Bag Syndrome



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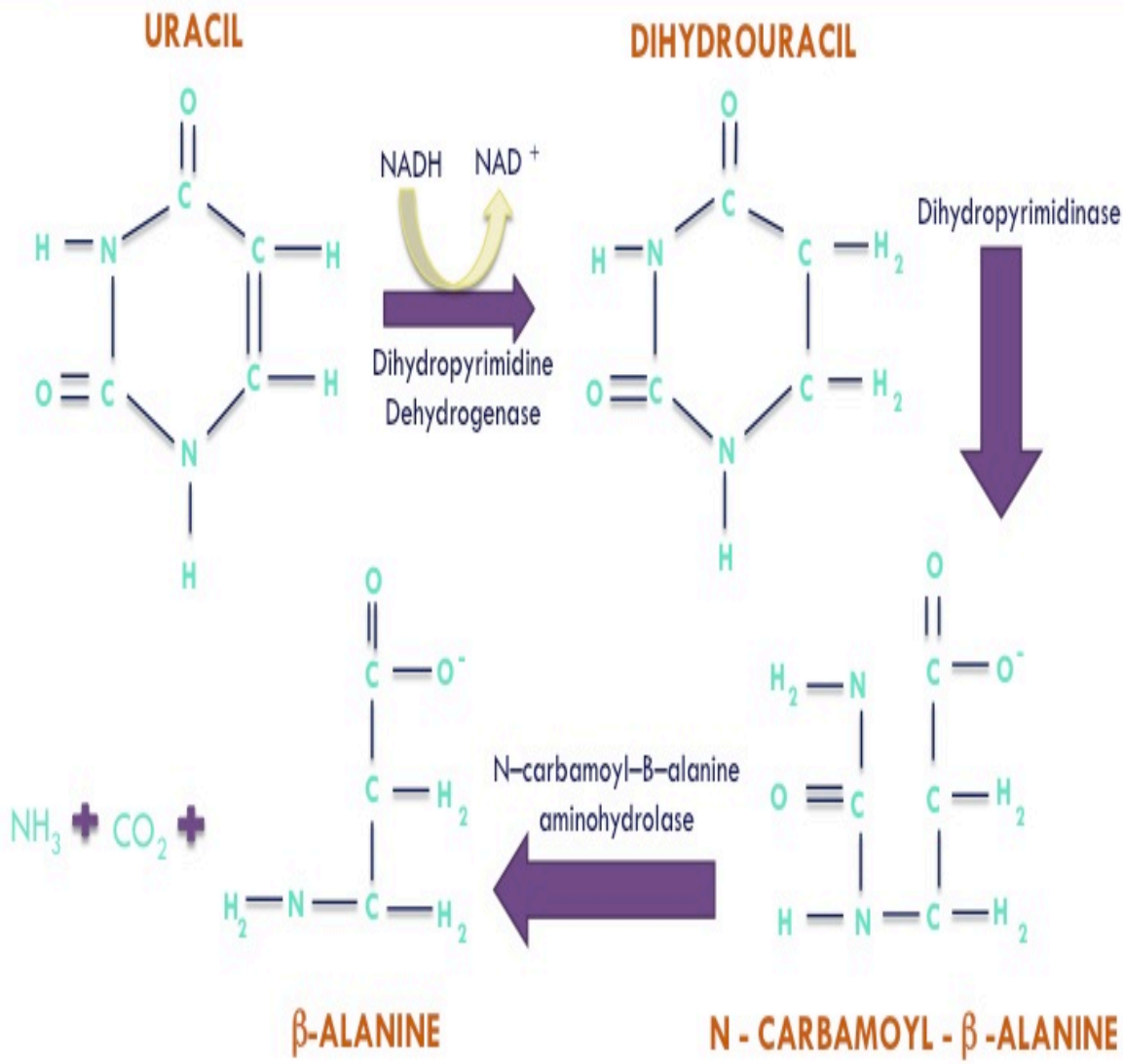
All organisms use metabolic processes to maintain homeostasis. These processes contain chemical reactions and can be divided into anabolism and catabolism. Anabolism, also called biosynthesis, requires the input of energy to create large, complex biomolecules from smaller, simpler subunits (Berg et al., 2008). Catabolism, also called degradation, breaks down large, complex biomolecules into its smaller components releasing energy (Berg et al., 2008). Metabolic processes are held in a dynamic equilibrium between their reactants and products. However, they require the use of proteins to catalyze each reaction. These proteins, enzymes, allow reactions to take place in physiological conditions by lowering the activation energy of the reaction (Voet et al., 2013). Enzymes are typically specific to one reaction, allowing them to catalyze the same reaction when additional reactants become available (Berg et al., 2008). Metabolic processes are organized into pathways, where the product of one reaction is used as the substrate in the next reaction until the desired biomolecule is made.

In this project, the first step in the pyrimidine degradative pathway was analyzed, which is typically considered to be the rate-limiting step (Voet et al., 2013). The entire degradative pathway can be seen in Figure 2. The enzyme in the first step of this pathway is Dihydropyrimidine Dehydrogenase, DHPD. This catalyzes the reduction of uracil and thymine to dihydrouracil and dihydrothymine, respectively. As the rate-limiting step in its pathway, this enzyme is highly regulated. This is by either feedback inhibition of the pathway's product or through the need of a cofactor

such as reduced nicotinamide adenine dinucleotide (NADH), making the study of this enzyme key to understanding the regulation of pyrimidines.

This project investigated Purple Urine Bag Syndrome to determine the cause of the unique discoloration in the urine. This project was to mimic the conditions that regulate a color change when simulating the urinary tract catheter in the laboratory. This research demonstrated what happened when these conditions were added to the metabolism to observe enzyme activity. Using the pyrimidine pathway, if a color was found, then the metabolic pathway could be regulated; therefore, regulation of the color occurred. This would result in the reduction of the patients' anxiety to avoid further health complications.

Figure 2. The Pyrimidine Degradative Reductive Pathway



Materials and Methods

Chemicals

Uracil, thymine, succinate, ethanol (200 proof), glutamate dehydrogenase, lysozyme, hydroxymethyl aminomethane (TRIS), sodium chloride, potassium bicarbonate, ketoglutaric acid (ketoglutarate), 3-uridopropanoic acid, N-carbamoyl-B-alanine (N-C-B-A), magnesium chloride, trichloroacetic acid, acetic acid, dihydrouracil, butadiene monoxime, antipyrine, nutrient broth, agar, Coomassie Brilliant Blue, phosphoric acid (85%), ammonium chloride, tryptophan, acetonitrile, hydrochloric acid, β-mercaptoethanol, and sulfuric acid were purchased from Fischer Scientific. Glucose, nicotinamide adenine dinucleotide (NADH), and nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from Sigma Aldrich.

Bacteria and Growth Media

The bacterium used in this study was *P. aeruginosa*, ATCC 15692. The cells were grown on nutrient agar plates then transferred to a liquid medium to allow the nutrients to become accustomed to the new environment. The liquid media was a modified Stanier minimal media containing potassium phosphate dibasic anhydrous (K_2HPO_4) (0.1% w/v), potassium phosphate monobasic (KH_2PO_4) (0.1% w/v), sodium chloride (NaCl) (0.1% w/v), Sodium Citrate (0.05% w/v), Magnesium Sulfate (0.07% w/v), a carbon source (0.4% w/v) and a nitrogen source (0.2% w/v) (Stanier, 1947). The nitrogen sources were uracil or thymine, while the carbon sources were glucose or succinate. Prior to sterilization, the pH of the medium was then adjusted to

between 7.0 and 7.2. In order to prevent carmelization, the magnesium sulfate was added after sterilization.

The bacterial cells were grown in 50 mL cultures of the liquid media. These cultures were shaken at 200 revolutions/minute (r.p.m.) at 30°C for at least 24 hours or until the liquid media became cloudy. This was done due to that being the optimal conditions for the enzyme. The batch was then split into three flasks containing 50 mL of fresh liquid media in preparation for assay. These were monitored spectrophotmetrically at 600 nm to determine the generation time for each species by observing a doubling to ensure the mid-exponential phase of growth was reached.

Preparation of Cell Extracts

After observing a growth doubling in the cells, they were processed to disrupt the cell membrane to release the enzymes. The cells were first collected by centrifugation at 10,400*g* for twenty minutes at 4°C. The liquid was then decanted and the pellets were washed by re-suspending them in 0.85% saline, after which, the cells were centrifuged again at 10,400*g* for twenty minutes at 4°C. After decanting the liquid, the pellets were suspended in 5 mL dialysis buffer, which consisted of 20 mM Tris-HCl pH 7.5, 1 mM EDTA, and 1 mM 2-mercaptoethanol (Hacker, 2007; Santiago & West, 2002). The cell suspension was sonicated in thirty-second intervals for a total of four minutes for each cell suspension to lyse the cellular membrane and remove the protein. This solution was then centrifuged at 10,400 *g* for twenty minutes at 4°C. The cellular free extract was then placed in dialysis tubing and left overnight in dialysis buffer in order to further purify the protein. The dialysis tubing allowed

anything smaller than 14 kilo Daltons to move freely in and out of the bag. This resulted in the removing of small biological molecules that weren't the biomass. Biomass is heavy, approximately 100 kilo Daltons; therefore, remaining in the dialysis bag resulting in a partially purified product. After dialysis, the proteins were removed from the buffer and placed into test tubes to use for analysis.

Enzyme Assays

These experiments measured the first enzyme in the reductive pathway of pyrimidines in order to determine the specific activity in the presence of various concentrations of acetonitrile, tryptophan and ammonia. Each enzyme had four independent culture samples that were analyzed, with the first being the blank. The blank contained everything in the reaction mixture except for the starting material, which was replaced with water. The other three samples were used to generate a specific activity and standard deviation.

Dihydropyrimidine Dehydrogenase, the rate-limiting first enzyme, in the pyrimidine degradative reductive pathway, was assayed in a reaction mixture containing 0.1 mL 1M Tris-HCl buffer pH 7.5, 0.1 mL NADH or NADPH, 0.4 mL de-ionized water, 0.2 mL cell free extract, and 0.2 mL 5 mM uracil or thymine (Hacker, 2007). A 5mM uracil or thymine solution was used to initiate this reaction and the blank received de-ionized water instead of the uracil or thymine. The total volume for this assay was 1.0 mL. When various concentrations of acetonitrile, tryptophan and ammonia were added, the amount of de-ionized water was adjusted to keep the 1.0 mL total volume.

The conversion of NADH to NAD^+, or NADPH to $NADP^+$, was measured over a 9.5-minute period, at a wavelength of 340 nm, and at 30°C. The molar absorptivity, 6.22 x 10^3 $M^{-1}cm^{-1}$ (French et al., 1997) of the cofactor was then utilized to calculate the specific activity of DHPD. The specific activity was reported in nanomoles per minute per milligram of protein.

Protein Assay

In order to determine the amount of protein in each sample assayed for specific protein, the Bradford protein assay was performed. The Bradford method utilizes a 1 mg/mL lysozyme standard and Bradford dye, which binds non-specifically to the protein. The Bradford dye was prepared by dissolving 0.01% (w/v) Coomassie Brilliant Blue G-250, 95% (w/v) ethanol, and 8.5% (w/v) *o*-phosphoric acid in de-ionized water (Bradford, 1976). A 4 mL aliquot was added to the diluted samples and then measured spectrophotometrically at 595 nm where the Bradford dye binds non-specifically to the protein. Each enzyme had three independent culture samples that were analyzed in order to produce a standard deviation. The lysozyme containing standards are then used to produce a standard curve, which was used to calculate the amount of protein in each assayed sample (Han et al., 2010).

Results

The objective of this research was to investigate possible contributes to Purple Urine Bag Syndrome. Effects of changes in environmental conditions were monitored by the specific activity of the first enzyme of the degradative reductive pathway of *P. aeruginosa* ATCC 15692 in optimal and minimal growth conditions. This project aimed to assess any changes in specific activity in the presence of 2 mM and 10 mM acetonitrile, 0.2 mM and 1.0 mM tryptophan, and 2 mM and 20 mM ammonia. The concentrations of these compounds were chosen in order to demonstrate the changes in specific activity with a more diluted concentration and a higher concentration. They were also chosen based on the normal levels of these compounds in healthy individuals (Huether et al., 1999; Putnam, 1971).

When *P. aeruginosa* ATCC 15692 cells were grown in minimal media containing glucose or succinate as the carbon source and uracil or thymine as the nitrogen source, generation times were found (Table 1). It can be seen that the generation times varied dependent on the nitrogen source and not the carbon source. When the nitrogen source was changed from uracil to thymine, the generation time, for both glucose and succinate as the carbon source, increased 2-fold.

Table 1. Generation Times of *P. aeruginosa* grown in Glucose or Succinate and Uracil or Thymine

Glucose Uracil (minutes)	Glucose Thymine (minutes)	Succinate Uracil (minutes)	Succinate Thymine (minutes)
211 ± 59	411 ± 47	189 ± 12	326 ± 57

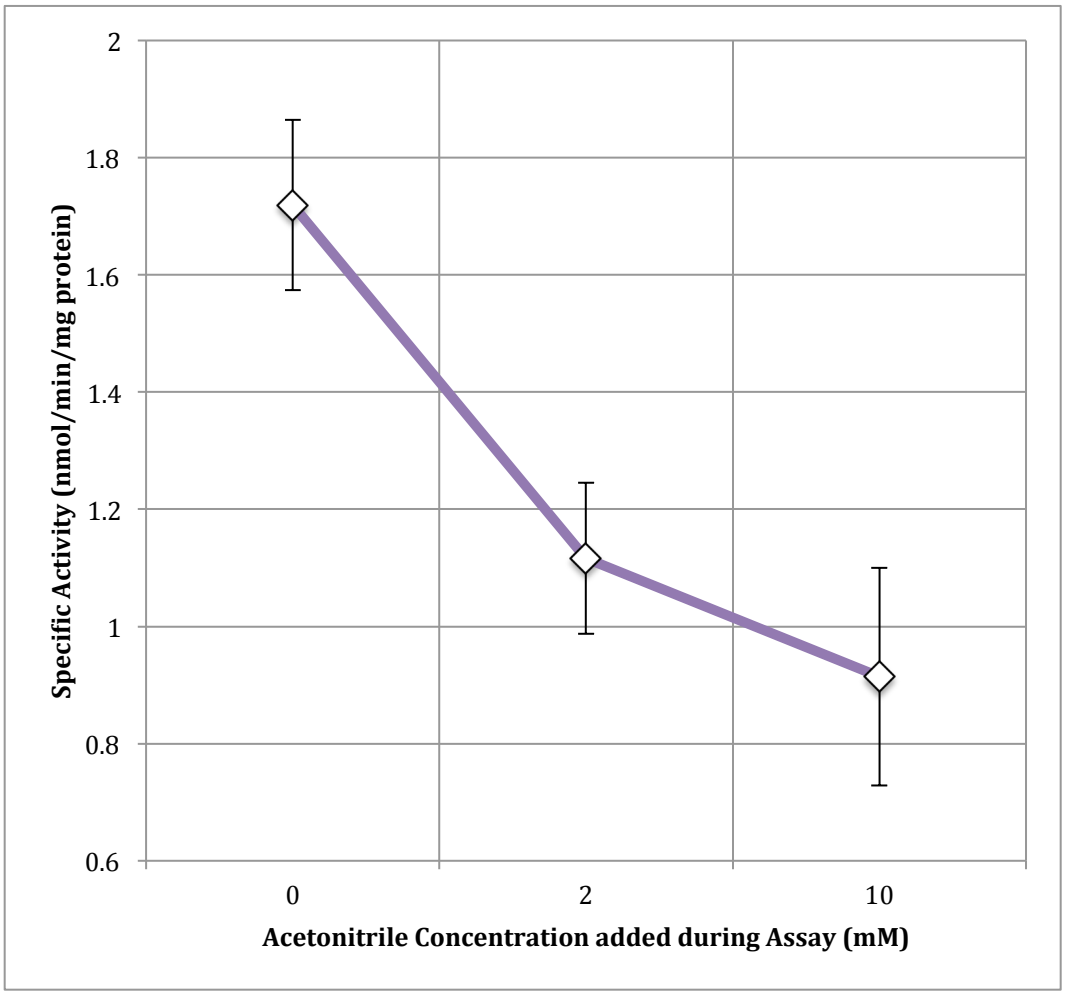
In order to determine the effects of the addition of acetonitrile, tryptophan and ammonia the dihydropyrimidine dehydrogenase (DHPD) assay was performed. This assay was performed on each of the cell-free extracts in Table 1. The effects of adding acetonitrile to glucose uracil minimal media can be seen in Table 2 and Figure 3. In Table 2, it can be seen that a 10-fold increase in acetonitrile concentration demonstrated a 2-fold decrease in specific activity. The effects of adding tryptophan to glucose uracil minimal media can be seen in Table 3 and Figure 4. In Table 3, the increase of tryptophan concentration by 5-fold showed a 1-fold increase when only adding tryptophan, a 1.5-fold increase when adding tryptophan and 2 mM acetonitrile, and a 3-fold decrease when adding tryptophan and 10 mM acetonitrile.

Table 2. Glucose Uracil Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	1.72 ± 0.145
2	1.12 ± 0.129
10	0.914 ± 0.186

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 3. *P. aeruginosa* grown in Glucose Uracil Minimal Media adding Acetonitrile



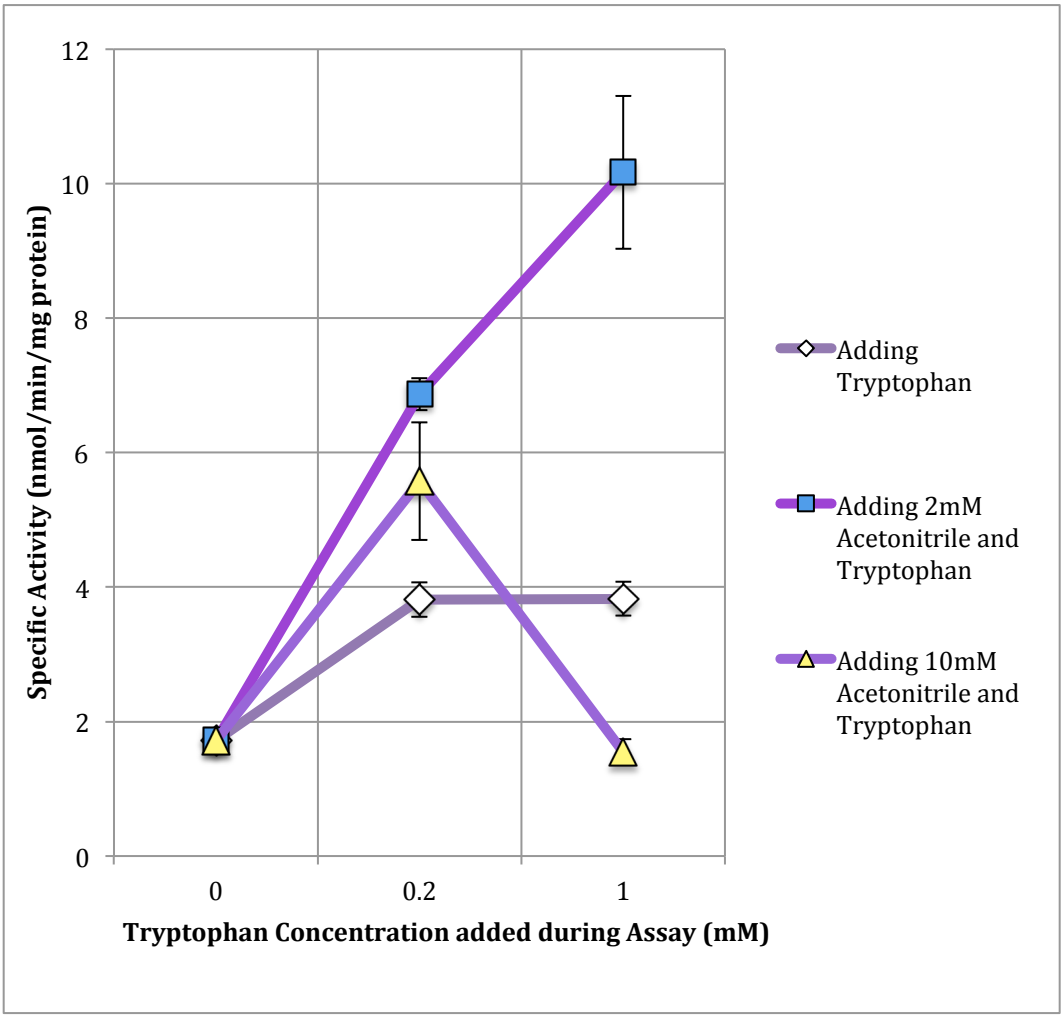
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen and 0.4% glucose as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 3. Glucose Uracil Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	3.81 $\pm$ 0.255	6.87 $\pm$ 0.236	5.58 $\pm$ 0.875
1.0	3.83 $\pm$ 0.251	10.2 $\pm$ 1.14	1.55 $\pm$ 0.189

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 4. *P. aeruginosa* grown in Glucose Uracil Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen and 0.4% glucose as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

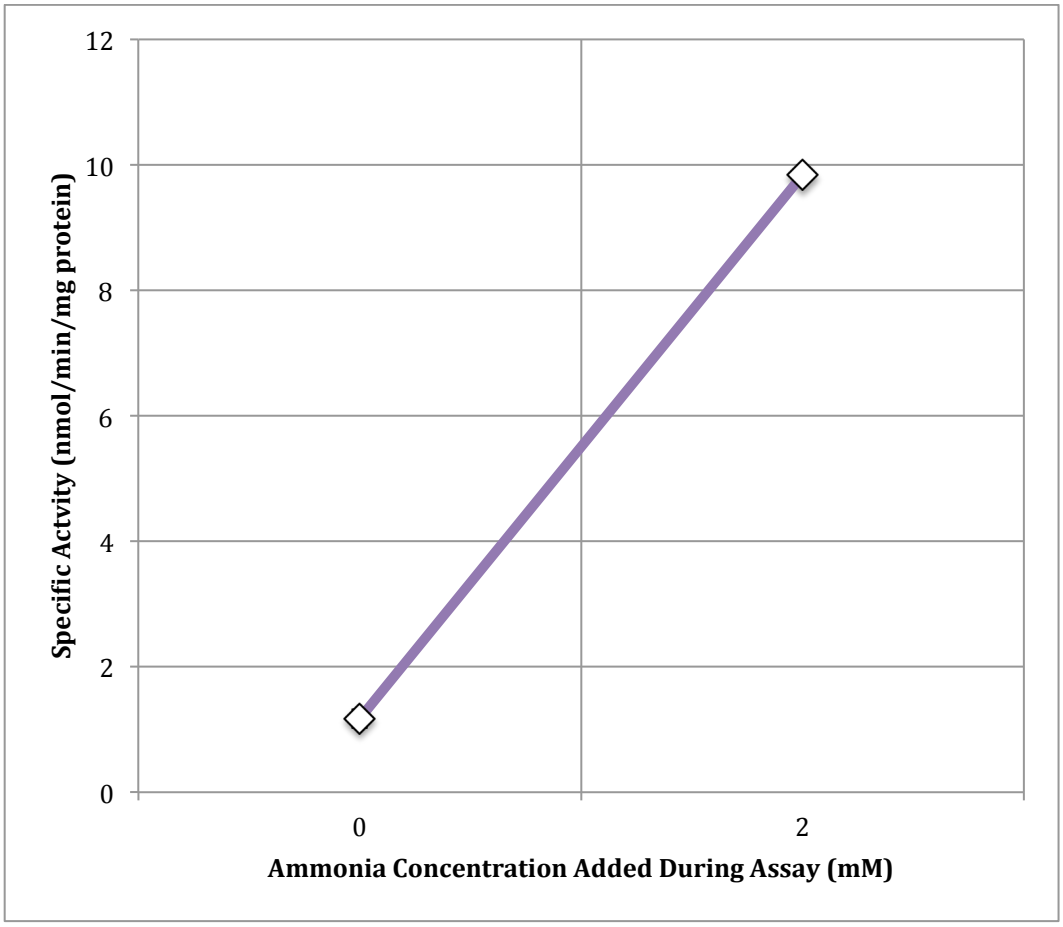
The effects of adding ammonia to glucose uracil minimal media can be seen in Table 4 and Figure 5. Table 4 shows that a 2-fold increase in ammonia concentration demonstrated a 9-fold increase in specific activity. The effects of then adding 2 mM ammonia and acetonitrile concentrations can be seen in Table 5 and Figure 6. A 2-fold increase in acetonitrile concentration showed a 2-fold increase in specific activity, whereas, a 10-fold increase in acetonitrile concentration showed a 1.5-fold decrease in specific activity. Table 6 and Figure 7 show the effects of adding 2mM ammonia, 2mM and 10mM acetonitrile, and tryptophan concentrations. When adding 2mM ammonia and tryptophan, a 5-fold increase in tryptophan concentration resulted in a 2-fold decrease in specific activity. When adding 2 mM ammonia, 2 mM acetonitrile and tryptophan, a 5-fold increase in tryptophan concentration resulted in a 2.7-fold increase in specific activity. When adding 2 mM ammonia, 10 mM acetonitrile and tryptophan, a 5-fold increase in tryptophan concentration resulted in a 3.3-fold decrease in specific activity.

Table 4. Glucose Uracil Minimal Media adding Ammonia

Ammonia Concentration (mM)	Adding Ammonia Concentrations Specific Activity (nmol/min/mg protein)
0	1.7 ± 0.145
2	9.84 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 5. *P. aeruginosa* grown in Glucose Uracil Minimal Media adding Ammonia



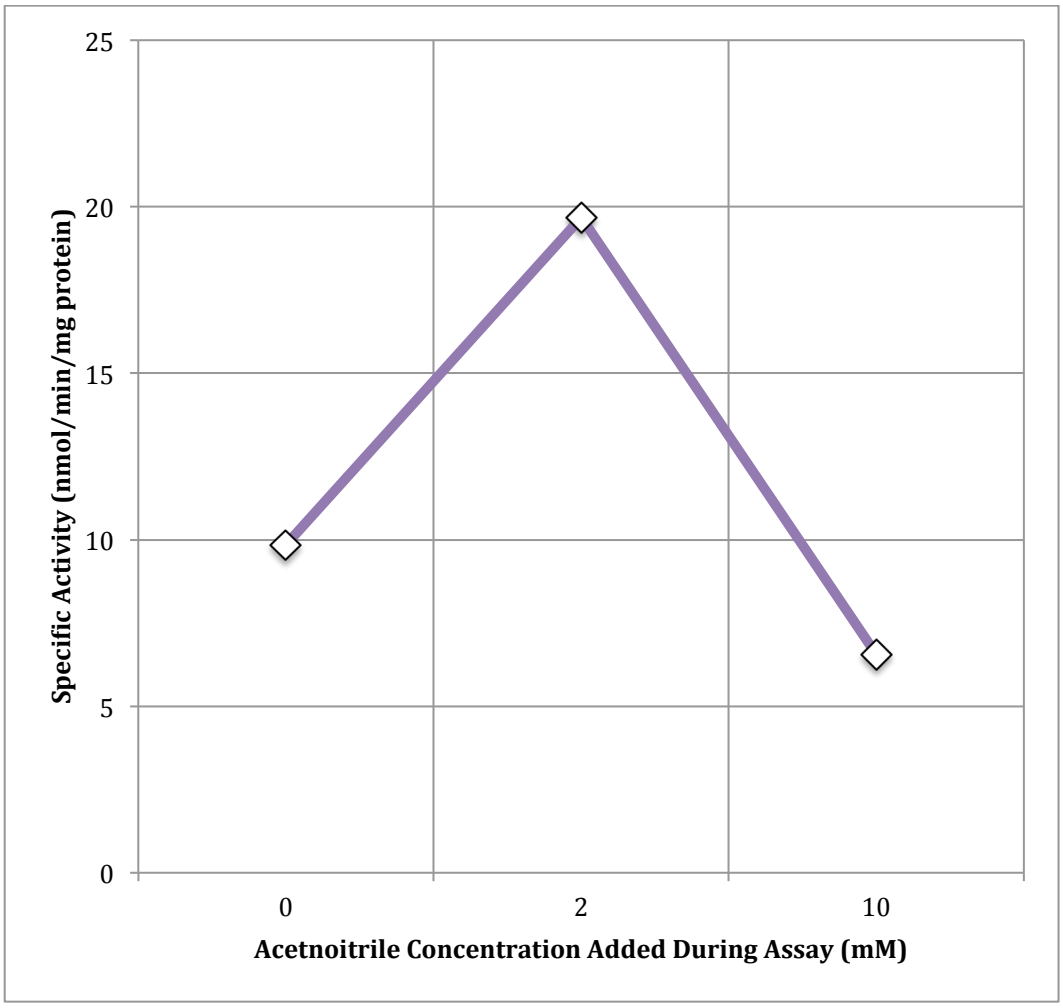
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen and 0.4% glucose as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the ammonia concentration. The values were an average of the values [standard deviation].

Table 5. Glucose Uracil Minimal Media adding 2mM Ammonia and Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	9.84 ± 0.000
2	19.7 ± 0.000
10	6.56 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 6. *P. aeruginosa* grown in Glucose Uracil Minimal Media adding 2mM Ammonia and Acetonitrile



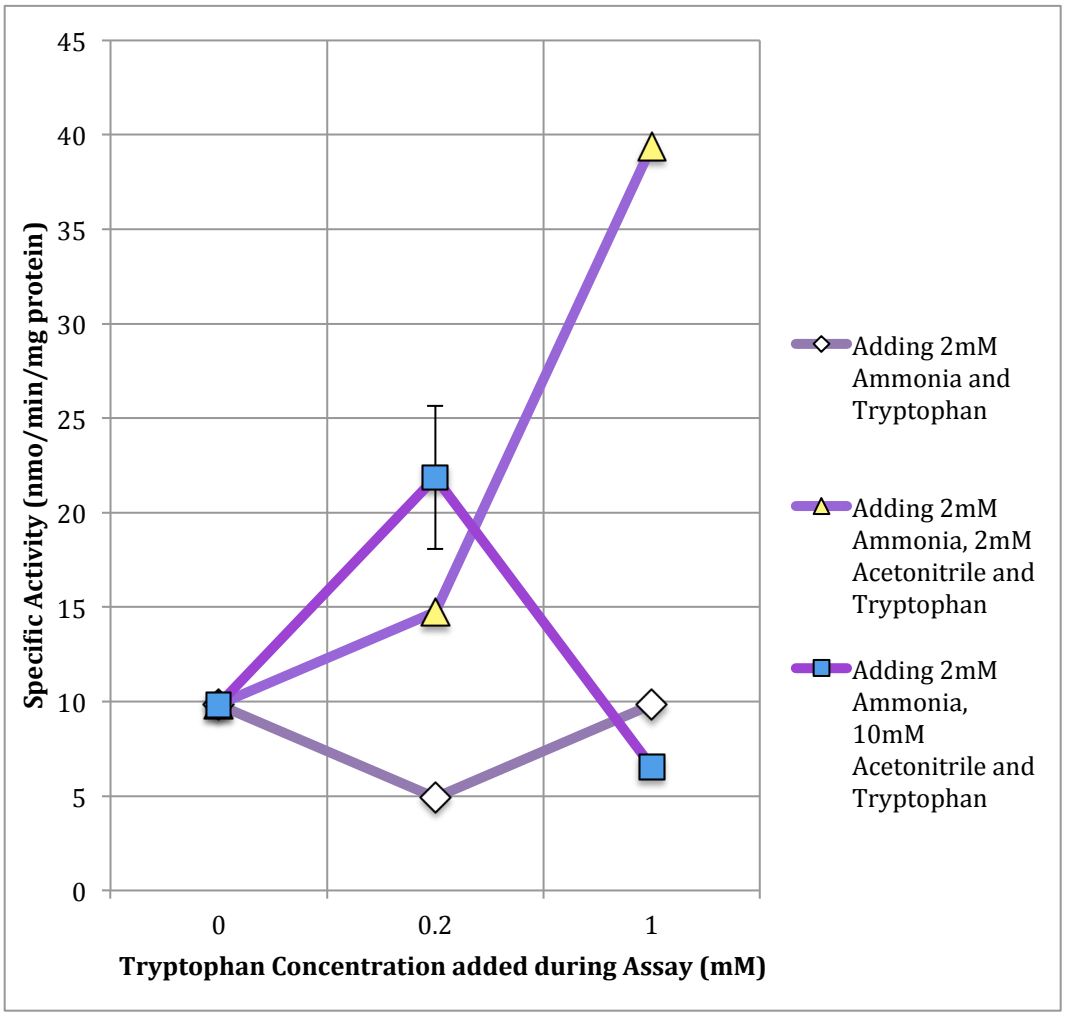
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen and 0.4% glucose as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 6. Glucose Uracil Minimal Media adding 2mM Ammonia, Acetonitrile and Tryptophan

Tryptophan Concentration (mM)	Adding 2mM Ammonia and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Ammonia, 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Ammonia, 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	4.92 $\pm$ 0.000	14.8 $\pm$ 0.000	21.9 $\pm$ 3.79
1.0	9.84 $\pm$ 0.000	39.4 $\pm$ 0.000	6.56 $\pm$ 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 7. *P. aeruginosa* grown in Glucose Uracil Minimal Media adding 2mM Ammonia, Acetonitrile and Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen and 0.4% glucose as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

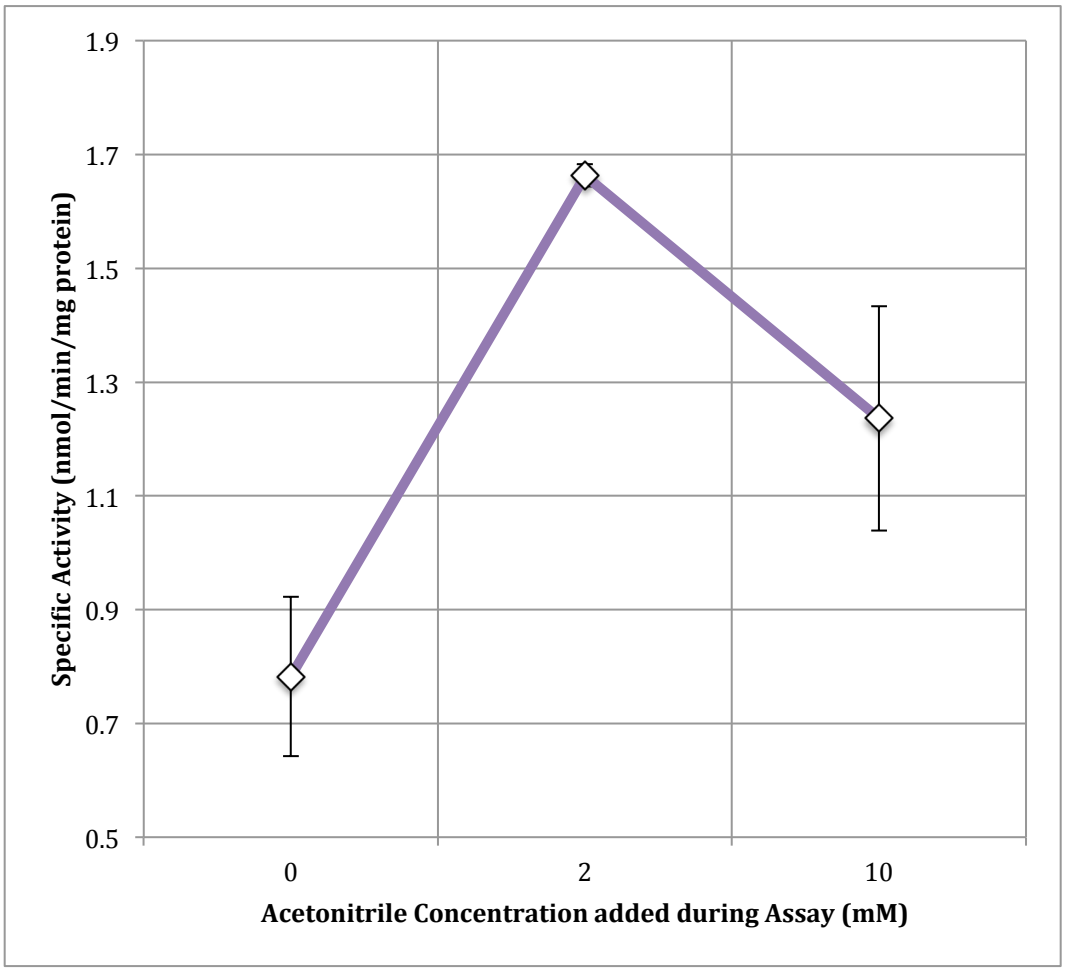
Table 7 and Figure 8 show the effects of adding acetonitrile to succinate uracil minimal media. A 2-fold increase in acetonitrile showed a 2-fold increase in specific activity, but a 10-fold increase in acetonitrile only showed a 1.5-fold increase in specific activity and this can be seen in Table 7. The effects of adding tryptophan to succinate uracil minimal media can be seen in Table 8 and Figure 9. It can be seen in Table 8 that a 5-fold increase of tryptophan concentration showed a 2-fold increase when only adding tryptophan, 1.2-fold increase when adding tryptophan and 2 mM acetonitrile, and a 2.7-fold decrease when adding tryptophan and 10 mM acetonitrile.

Table 7. Succinate Uracil Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.782 ± 0.140
2	1.66 ± 0.0194
10	1.24 ± 0.197

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 8. *P. aeruginosa* grown in Succinate Uracil Minimal Media adding Acetonitrile



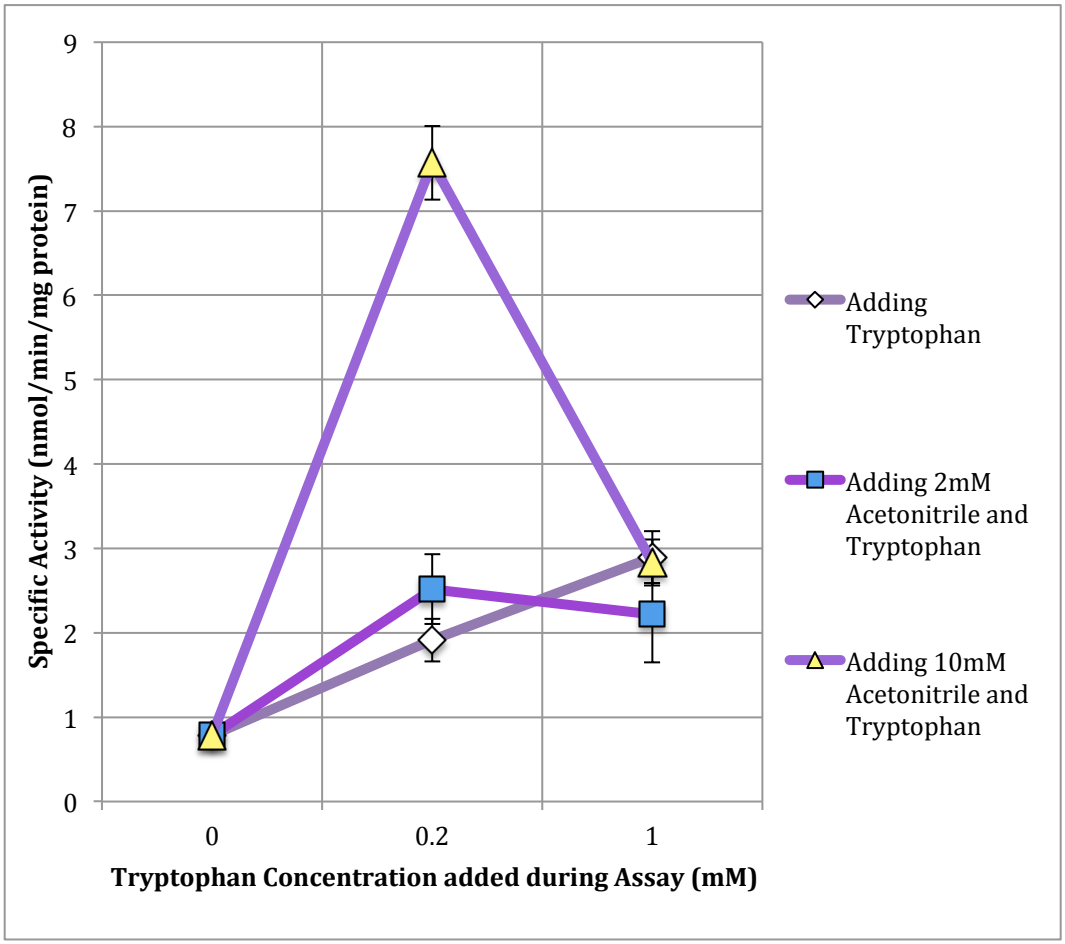
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen and 0.4% succinate as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 8. Succinate Uracil Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	1.91 ± 0.251	2.52 ± 0.416	7.57 ± 0.437
1.0	2.90 ± 0.307	2.22 ± 0.569	2.83 ± 0.271

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 9. *P. aeruginosa* grown in Succinate Uracil Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen and 0.4% succinate as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30°C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

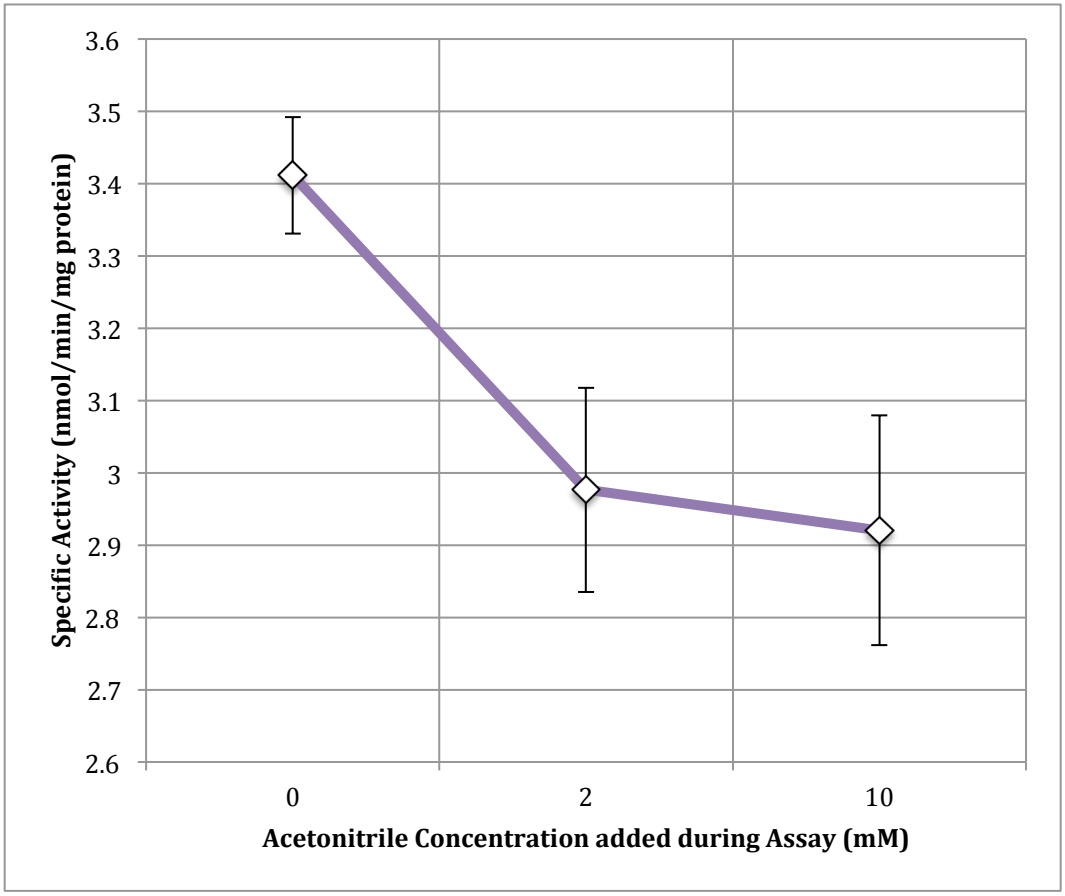
The effects of adding acetonitrile to glucose thymine minimal media can be seen in Table 9 and Figure 10. Table 9 shows that a 2-fold and 10-fold increase in acetonitrile concentration showed a 1.2-fold decrease in specific activity. Table 10 and Figure 11 show the effects of adding tryptophan to glucose thymine minimal media. It can be seen in Table 10 that a 5-fold increase in tryptophan concentration showed a 2.6-fold increase in specific activity when only tryptophan was added, no increase in specific activity when tryptophan and 2 mM acetonitrile was added, and a 1.8-fold decrease in specific activity when tryptophan and 10 mM acetonitrile was added.

Table 9. Glucose Thymine Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	3.41 ± 0.0809
2	2.98 ± 0.141
10	2.92 ± 0.159

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 10. *P. aeruginosa* grown in Glucose Thymine Minimal Media adding Acetonitrile



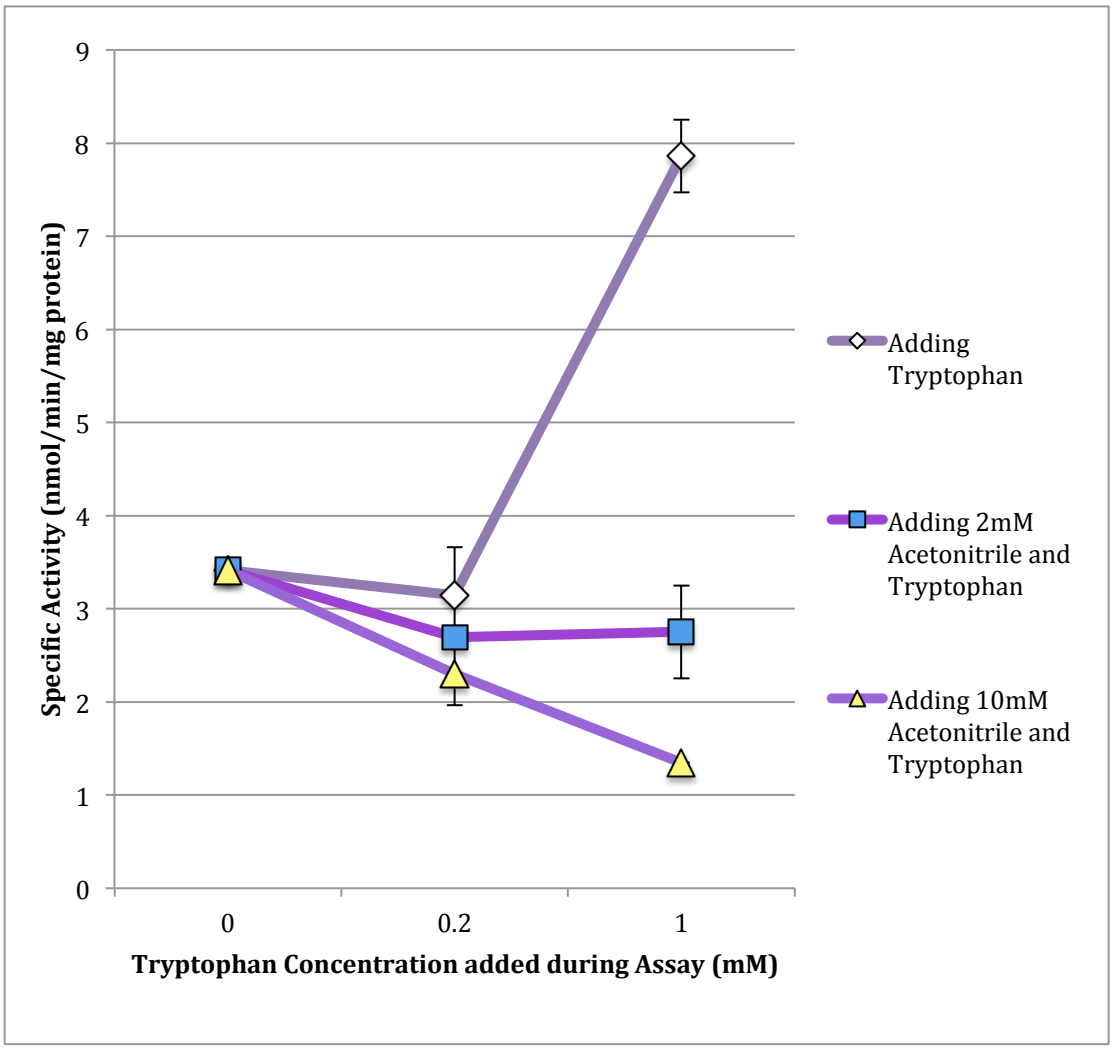
These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen and 0.4% glucose as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 10. Glucose Thymine Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	3.15 ± 0.515	2.70 ± 0.000	2.30 ± 0.337
1.0	7.86 ± 0.389	2.75 ± 0.498	1.35 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 11. *P. aeruginosa* grown in Glucose Thymine Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen and 0.4% glucose as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

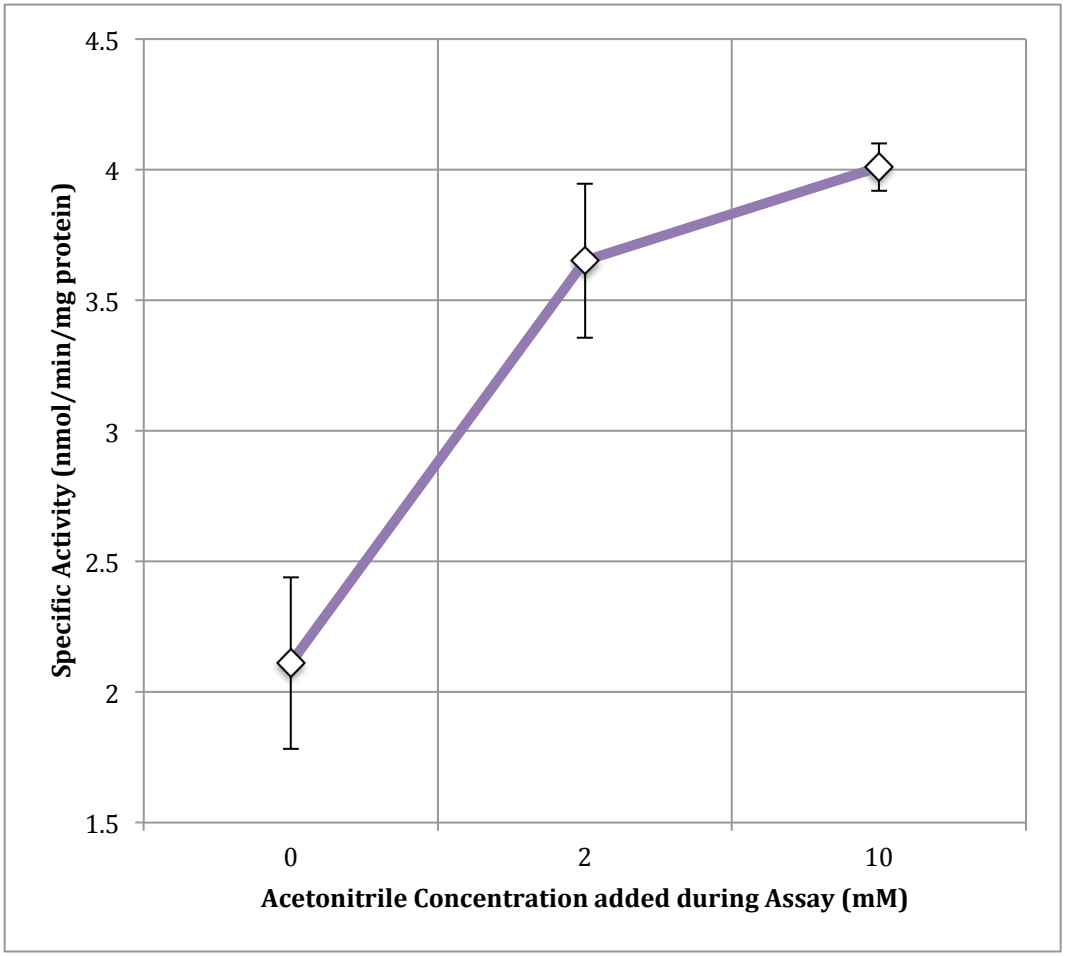
In Table 11 and Figure 12, the effects of adding acetonitrile to succinate thymine minimal media can be seen. Table 11 shows that a 10-fold increase in acetonitrile concentration showed a 2-fold increase in specific activity. The effects of adding tryptophan to succinate thymine minimal media can be seen in Table 12 and Figure 13. Table 12 shows a 5-fold increase in tryptophan concentration showed a 1.4-fold increase in specific activity when only adding tryptophan, 2.75-fold decrease in specific activity when adding tryptophan and 2 mM acetonitrile, and a 2-fold increase in specific activity when adding tryptophan and 10mM acetonitrile.

Table 11. Succinate Thymine Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	2.11 ± 0.328
2	3.65 ± 0.295
10	4.01 ± 0.0906

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 12. *P. aeruginosa* grown in Succinate Thymine Minimal Media adding Acetonitrile



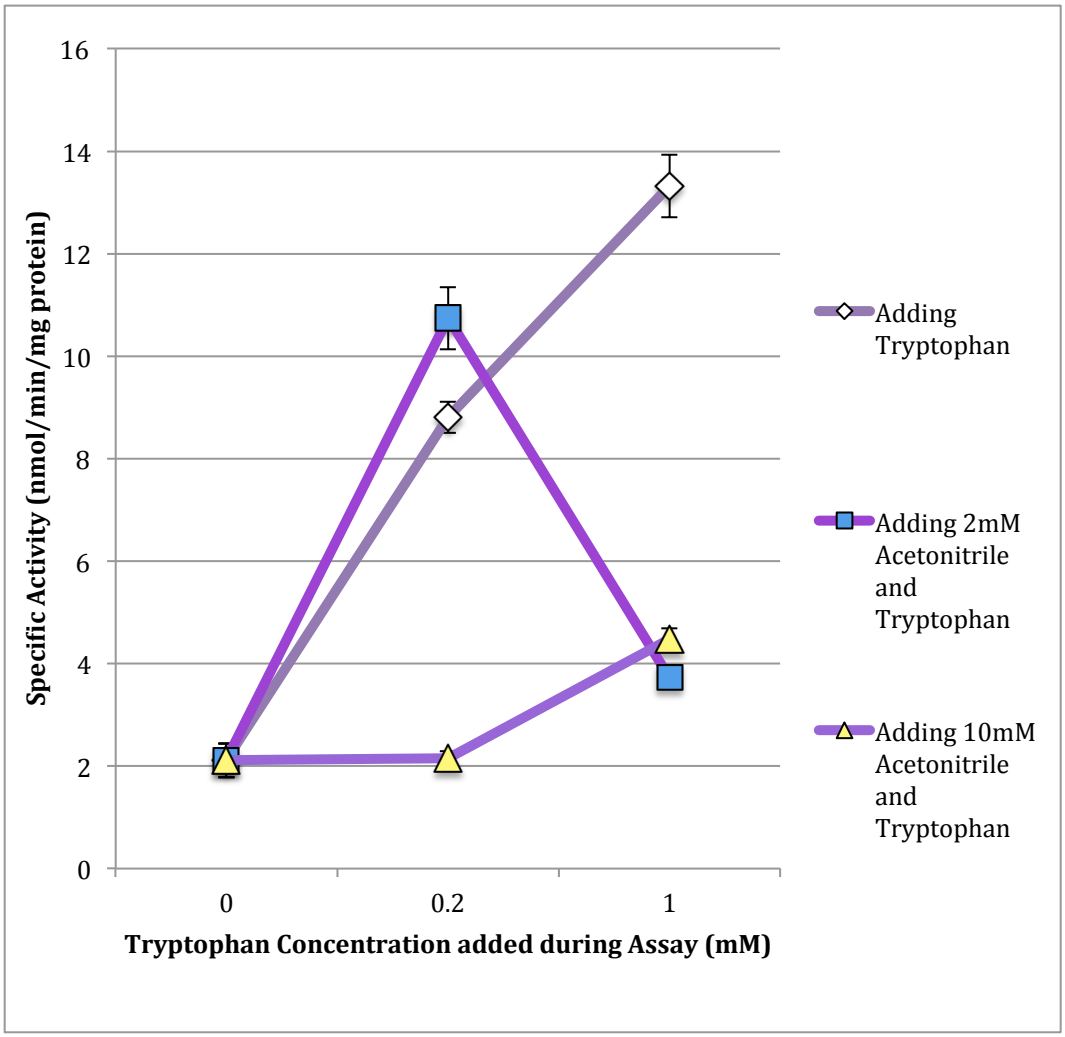
These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen and 0.4% succinate as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 12. Succinate Thymine Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	8.81 ± 0.304	10.7 ± 0.608	2.15 ± 0.143
1.0	13.3 ± 0.608	3.72 ± 0.000	4.48 ± 0.215

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 13. *P. aeruginosa* grown in Succinate Thymine Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen and 0.4% succinate as the carbon source. Specific activity was expressed as nanomoles/minute/milligram of protein at 30°C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

When the cells were grown in minimal media containing glucose or succinate as the carbon source, uracil or thymine as the nitrogen source and 2 mM or 10 mM concentrations of acetonitrile generation times were found (Table 13 and Table 14). It can be seen with these minimal media that the generation times varied dependent upon the nitrogen source within each carbon source. Glucose carbon source resulted in higher generation times than the succinate carbon source. When the nitrogen source was changed from uracil to thymine, using glucose as the carbon source with 2 mM acetonitrile, the generation time decreased by 1.3-fold. When the nitrogen source changed from uracil to thymine, using succinate as the carbon source with 2 mM acetonitrile, the generation time decreased by 2-fold. As the nitrogen source was changed from uracil to thymine, using glucose as the carbon source with 10 mM acetonitrile, the generation time decreased by 2.4-fold. Uracil being exchanged with thymine as the nitrogen source, using succinate as the carbon source with 10 mM acetonitrile, the generation time decreased by 1.2 fold.

Table 13. Generation Times in Glucose or Succinate and Uracil or Thymine with 2 mM Acetonitrile

Glucose Uracil with 2mM Acetonitrile (minutes)	Glucose Thymine with 2mM Acetonitrile (minutes)	Succinate Uracil with 2mM Acetonitrile (minutes)	Succinate Thymine with 2mM Acetonitrile (minutes)
518 ± 101	390 ± 43	330 ± 71	160 ± 9

Table 14. Generation Times in Glucose or Succinate and Uracil or Thymine with 10 mM Acetonitrile

Glucose Uracil with 10mM Acetonitrile (minutes)	Glucose Thymine with 10mM Acetonitrile (minutes)	Succinate Uracil with 10mM Acetonitrile (minutes)	Succinate Thymine with 10mM Acetonitrile (minutes)
201 ± 72	85 ± 39	208 ± 59	169 ± 11

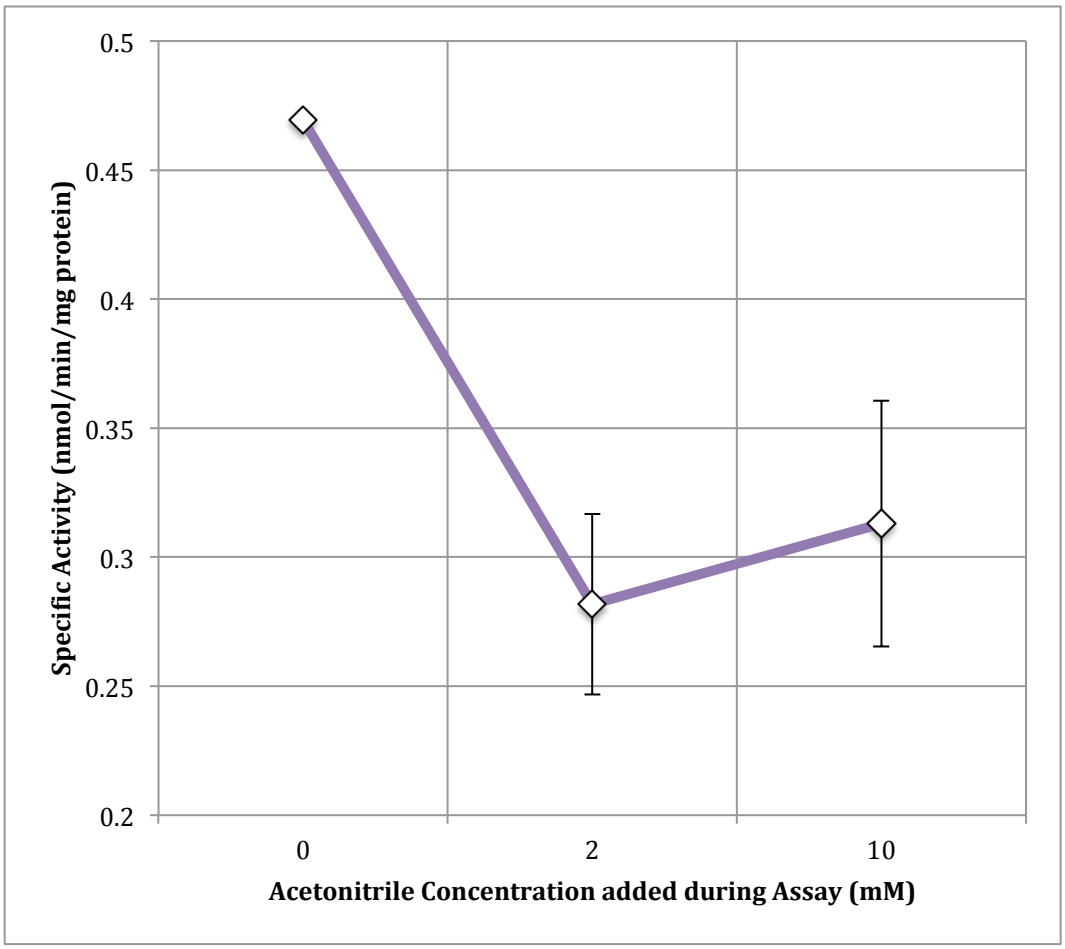
In order to determine the effects of the addition of acetonitrile, tryptophan and ammonia the dihydropyrimidine dehydrogenase (DHPD) assay was performed. This assay was performed on each of the cell-free extracts in Tables 13 and 14. The effects of adding acetonitrile to glucose uracil with 2mM acetonitrile minimal media can be seen in Table 15 and Figure 14. It can be seen in Table 15 that a 2-fold increase in acetonitrile concentration resulted in a 1.7-fold decrease in specific activity, but a 5-fold increase in acetonitrile concentration resulted in a 1.1-fold increase in specific activity. The effects of adding tryptophan to glucose uracil with 2 mM acetonitrile minimal media can be seen in Table 16 and Figure 15. It can be seen in Table 16 that a 5-fold increase in tryptophan concentration resulted in a 3.7-fold decrease when only adding tryptophan concentrations, a 1.9-fold increase when adding 2 mM acetonitrile and tryptophan, and a 1-fold increase when adding 10 mM acetonitrile and tryptophan.

Table 15. Glucose Uracil with 2 mM Acetonitrile Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.470 ± 0.000
2	0.282 ± 0.0350
10	0.313 ± 0.0476

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 14. *P. aeruginosa* grown in Glucose Uracil with 2 mM Acetonitrile Minimal Media adding Acetonitrile



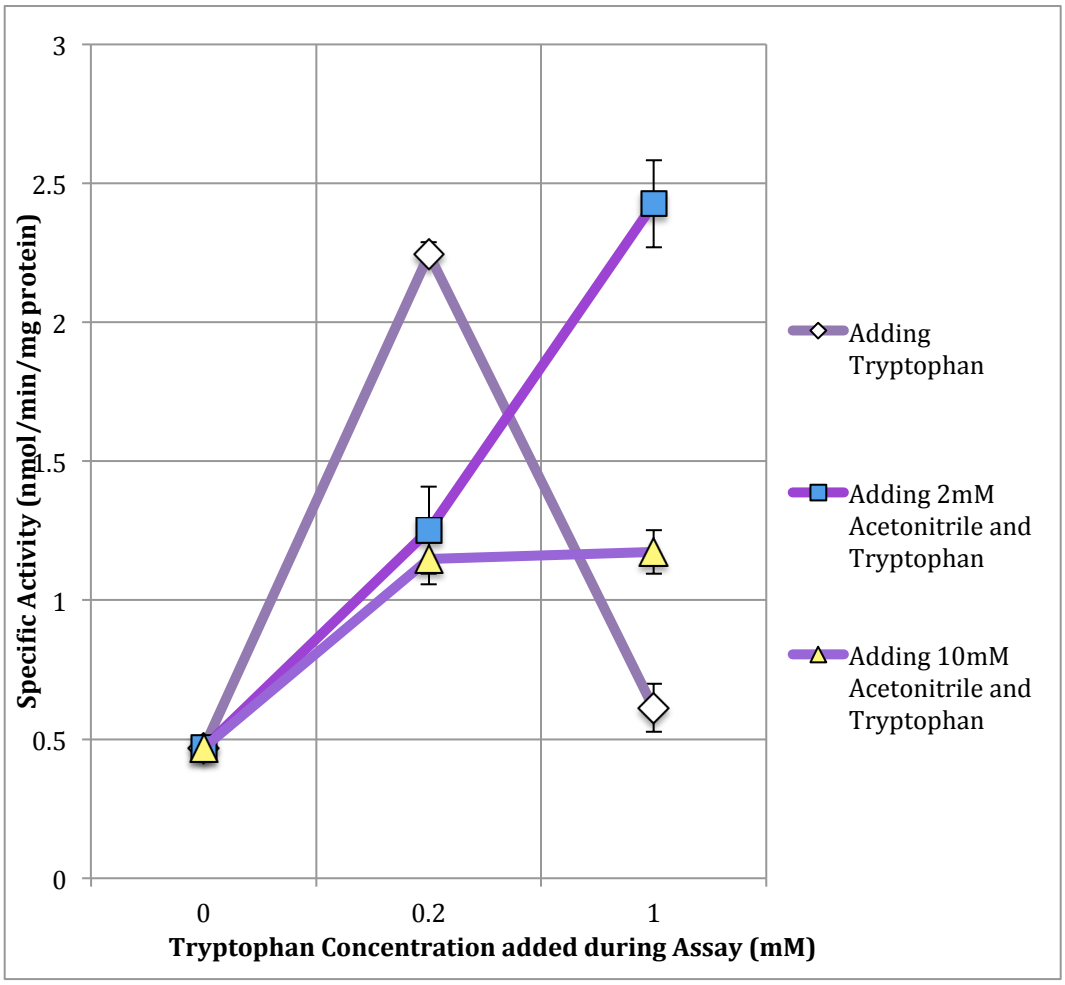
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, and 2 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 16. Glucose Uracil with 2 mM Acetonitrile Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	2.24 $\pm$ 0.0452	1.25 $\pm$ 0.157	1.15 $\pm$ 0.0904
1.0	0.613 $\pm$ 0.0863	2.43 $\pm$ 0.157	1.17 $\pm$ 0.0783

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 15. *P. aeruginosa* grown in Glucose Uracil with 2 mM Acetonitrile Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, and 2 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

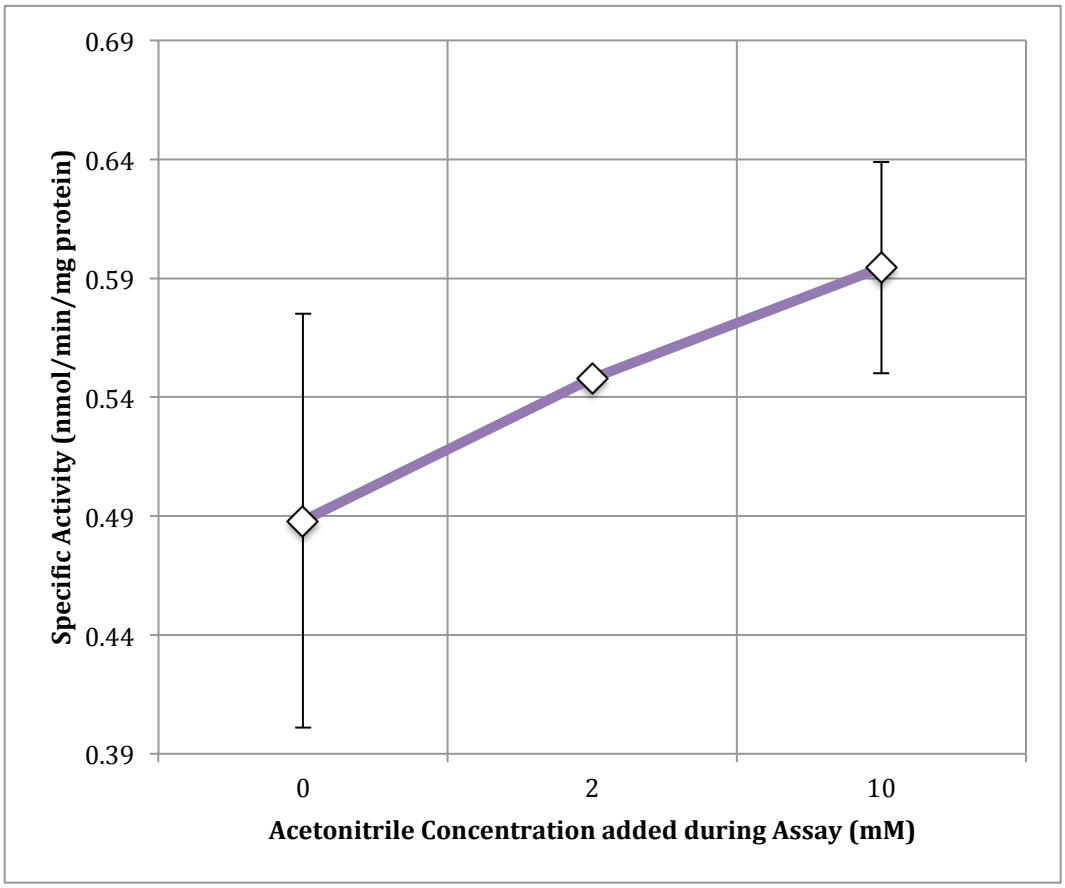
Adding acetonitrile to glucose uracil with 10 mM acetonitrile can be seen in Table 17 and Figure 16. In Table 17, it can be seen that a 2-fold increase in acetonitrile resulted in a 1.1-fold increase in specific activity and a 10-fold increase in acetonitrile resulted in only a 1.2-fold increase in specific activity. The effects of adding tryptophan to glucose uracil with 10 mM acetonitrile minimal media can be seen in Table 18 and Figure 17. It can be seen in Table 18 that a 5-fold increase in tryptophan concentration resulted in a 1.7-fold increase in specific activity when adding tryptophan concentrations, a 1.8-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 3.7-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 17. Glucose Uracil with 10 mM Acetonitrile Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.488 ± 0.0870
2	0.548 ± 0.000
10	0.594 ± 0.0444

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 16. *P. aeruginosa* grown in Glucose Uracil with 10 mM Acetonitrile Minimal Media adding Acetonitrile



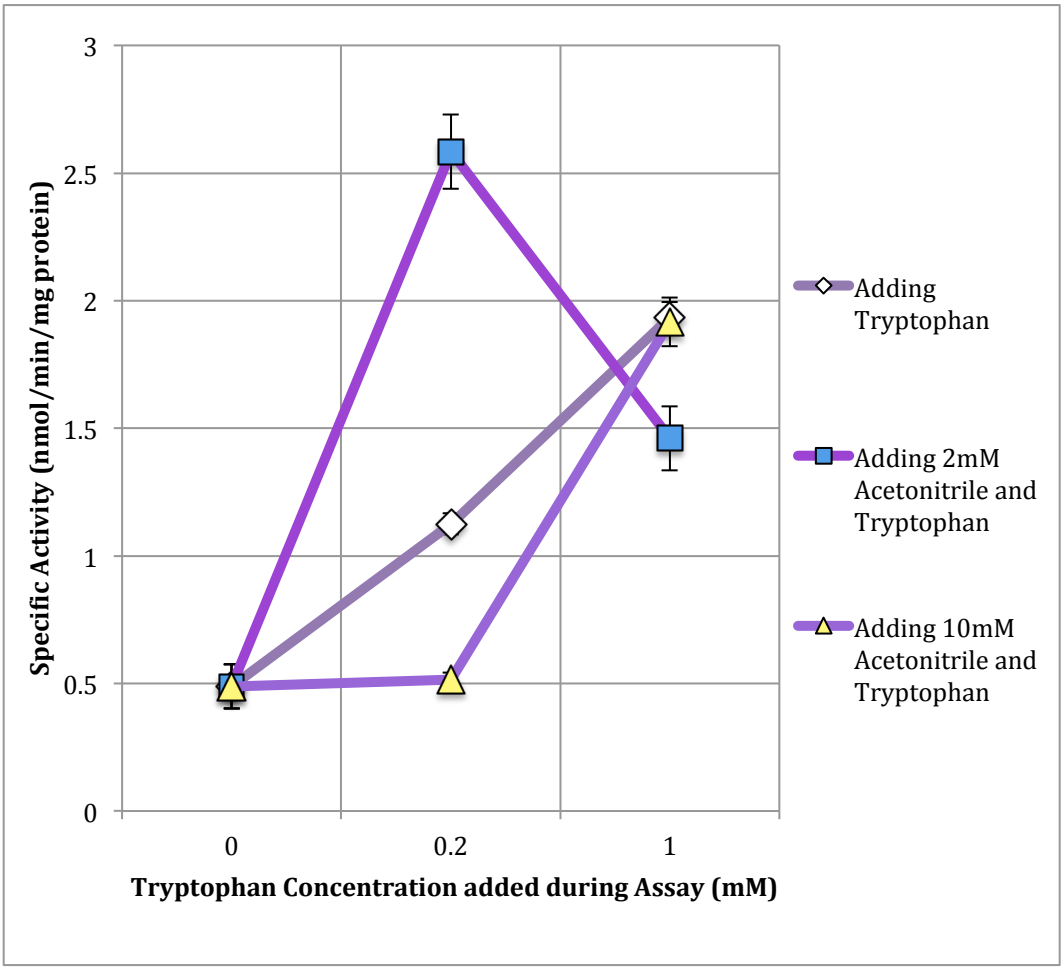
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, and 10 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 18. Glucose Uracil with 10 mM Acetonitrile Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	1.13 ± 0.0409	2.58 ± 0.146	0.515 ± 0.0288
1.0	1.93 ± 0.0626	1.46 ± 0.125	1.92 ± 0.0953

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 17. *P. aeruginosa* grown in Glucose Uracil with 10 mM Acetonitrile Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, and 10 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

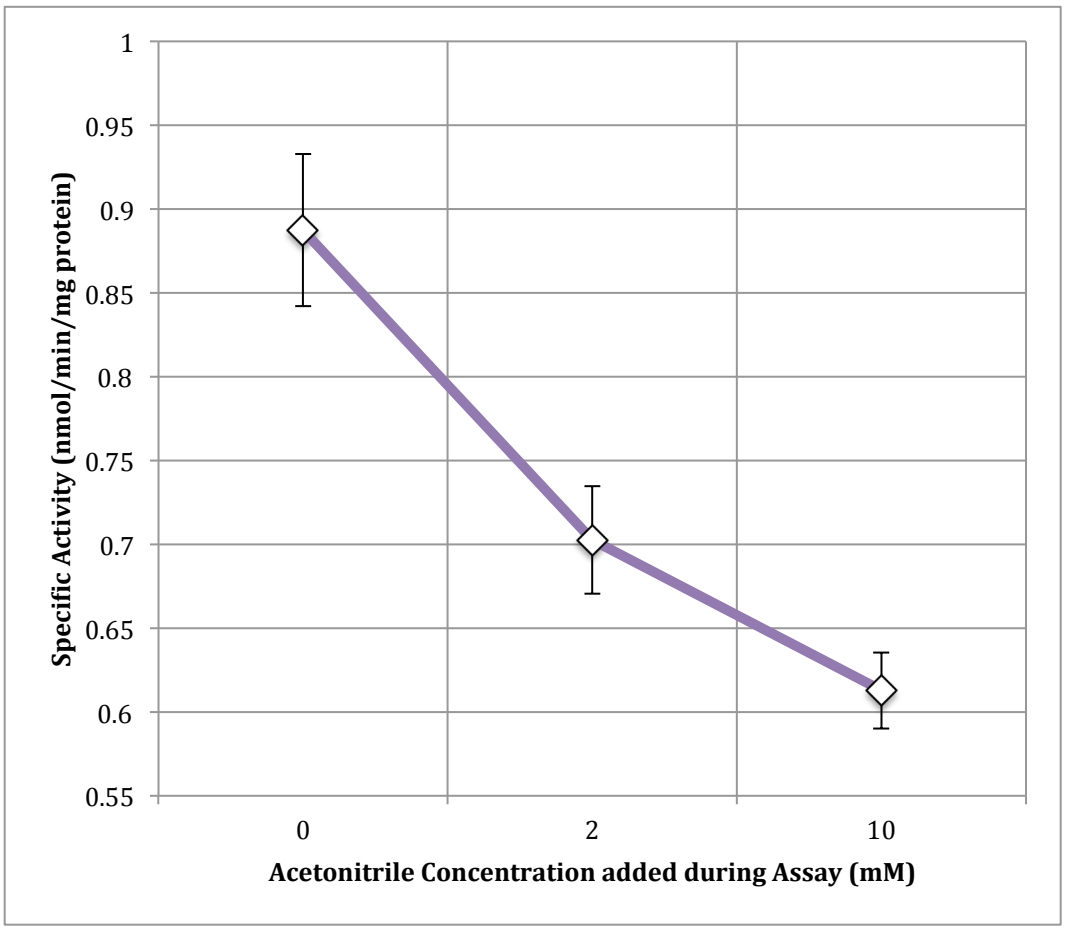
Table 19 and Figure 18 show the effects of adding acetonitrile to succinate uracil with 2 mM acetonitrile minimal media. A 2-fold increase in acetonitrile resulted in a 1.3-fold decrease in specific activity and a 10-fold increase in acetonitrile resulted in a 1.4-fold decrease in specific activity can be seen in Table 19. The effects of adding tryptophan to succinate uracil with 2 mM acetonitrile minimal media can be seen in Table 20 and Figure 19. It can be seen in Table 20 that a 5-fold increase in tryptophan concentration resulted in a 2.2-fold increase in specific activity when adding tryptophan concentrations, a 5.8-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.1-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 19. Succinate Uracil with 2 mM Acetonitrile Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.887 ± 0.0453
2	0.703 ± 0.0320
10	0.613 ± 0.0227

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 18. *P. aeruginosa* grown in Succinate Uracil with 2 mM Acetonitrile Minimal Media adding Acetonitrile



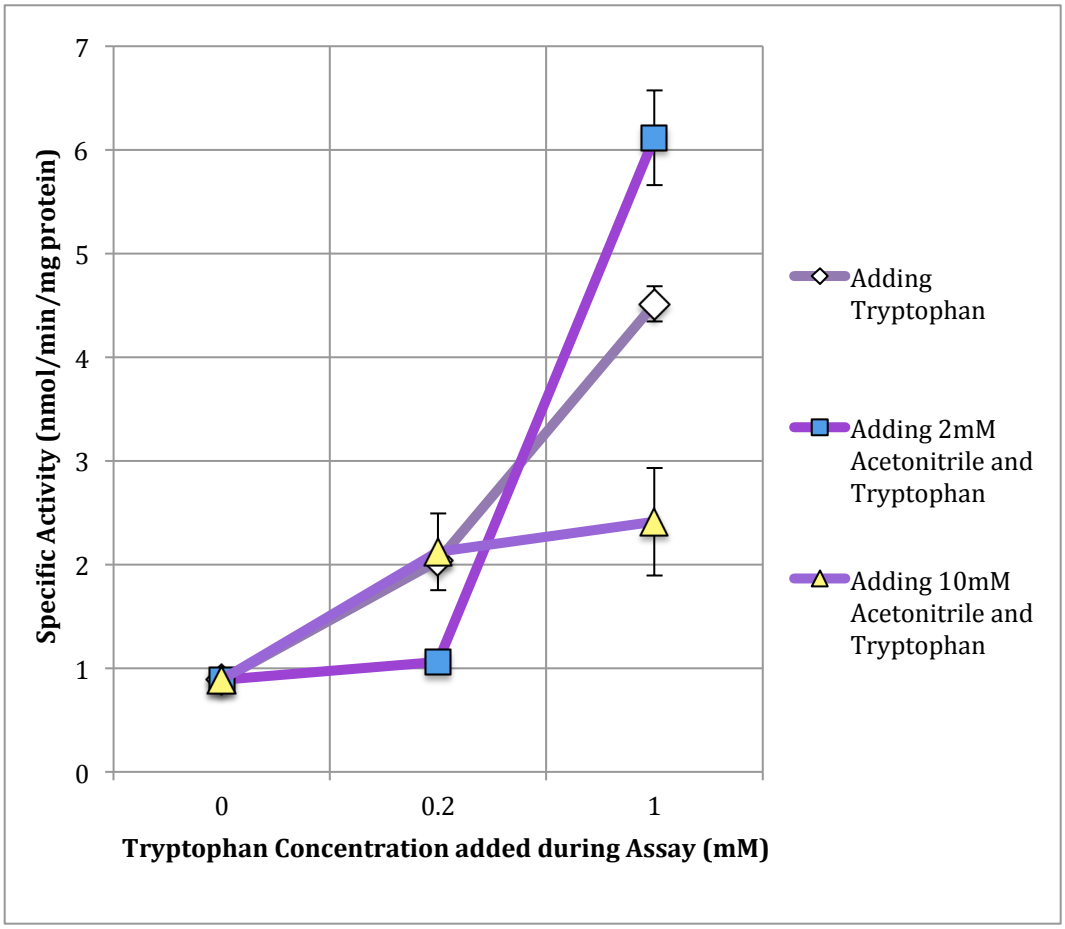
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% succinate as the carbon source, and 2 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 20. Succinate Uracil with 2 mM Acetonitrile Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	2.04 ± 0.0454	1.06 ± 0.0797	2.12 ± 0.369
1.0	4.51 ± 0.172	6.11 ± 0.458	2.41 ± 0.520

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 19. *P. aeruginosa* grown in Succinate Uracil with 2 mM Acetonitrile Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% succinate as the carbon source, and 2 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

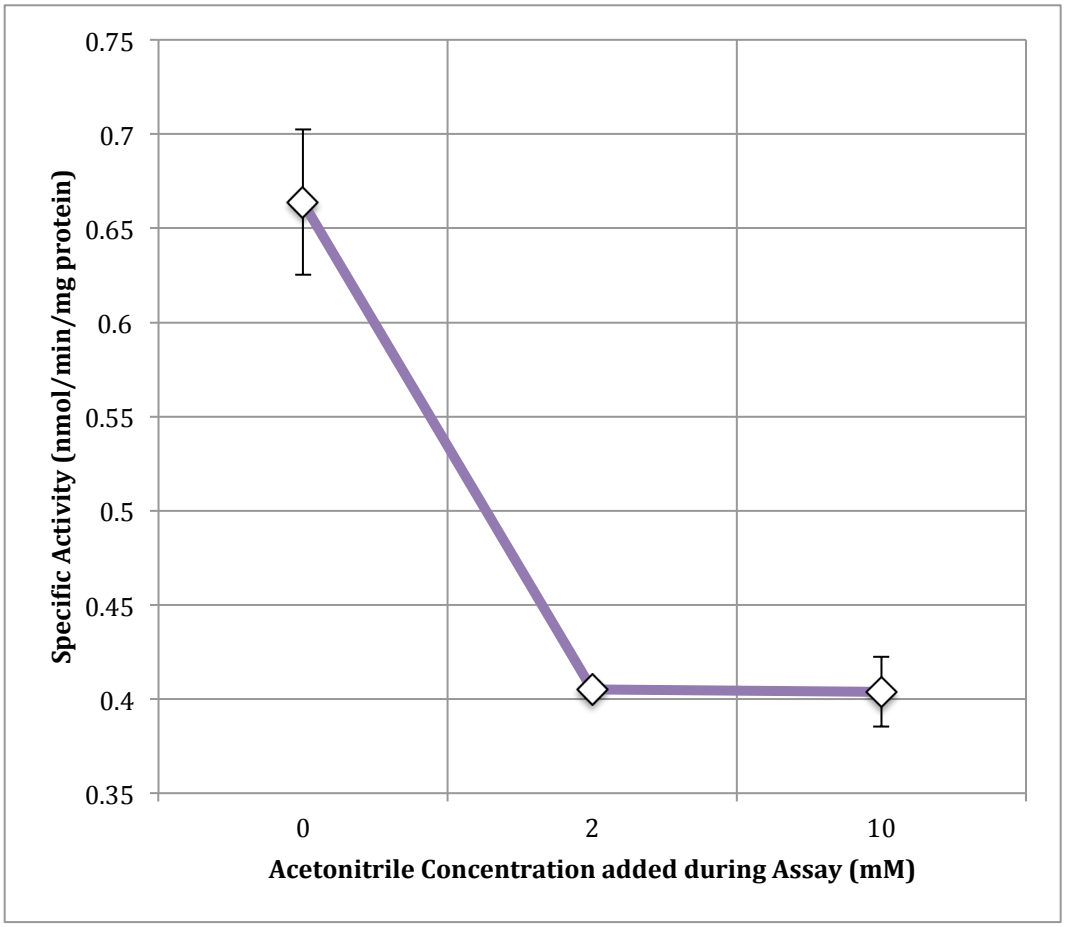
The effects of adding acetonitrile to succinate uracil with 10 mM acetonitrile minimal media can be seen in Table 21 and Figure 20. Table 21 shows that a 2-fold increase in acetonitrile concentration resulted in a 1.6-fold decrease in specific activity and a 10-fold increase in acetonitrile concentration also resulted in a 1.6-fold decrease in specific activity. Adding tryptophan to succinate uracil with 10 mM acetonitrile minimal media can be seen in Table 22 and Figure 21. It can be seen in Table 22 that a 5-fold increase in tryptophan concentration resulted in a 1.7-fold decrease in specific activity when adding tryptophan concentrations, a 1.1-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.7-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 21. Succinate Uracil with 10 mM Acetonitrile Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.664 ± 0.0386
2	0.405 ± 0.00428
10	0.404 ± 0.0185

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 20. *P. aeruginosa* grown in Succinate Uracil with 10 mM Acetonitrile Minimal Media adding Acetonitrile



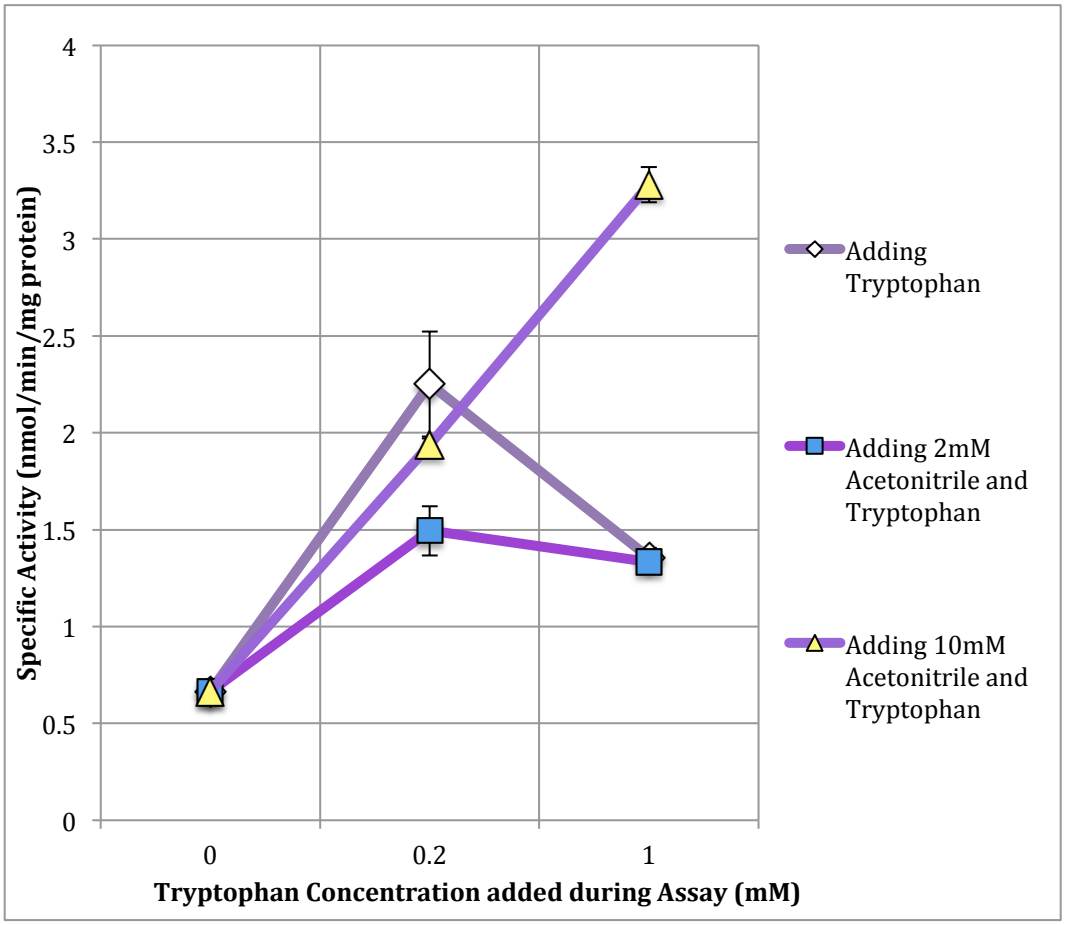
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% succinate as the carbon source, and 10 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 22. Succinate Uracil with 10 mM Acetonitrile Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	2.25 ± 0.272	1.49 ± 0.127	1.94 ± 0.0342
1.0	1.36 ± 0.0506	1.33 ± 0.0297	3.28 ± 0.0905

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 21. *P. aeruginosa* grown in Succinate Uracil with 10mM Acetonitrile Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% succinate as the carbon source, and 10 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

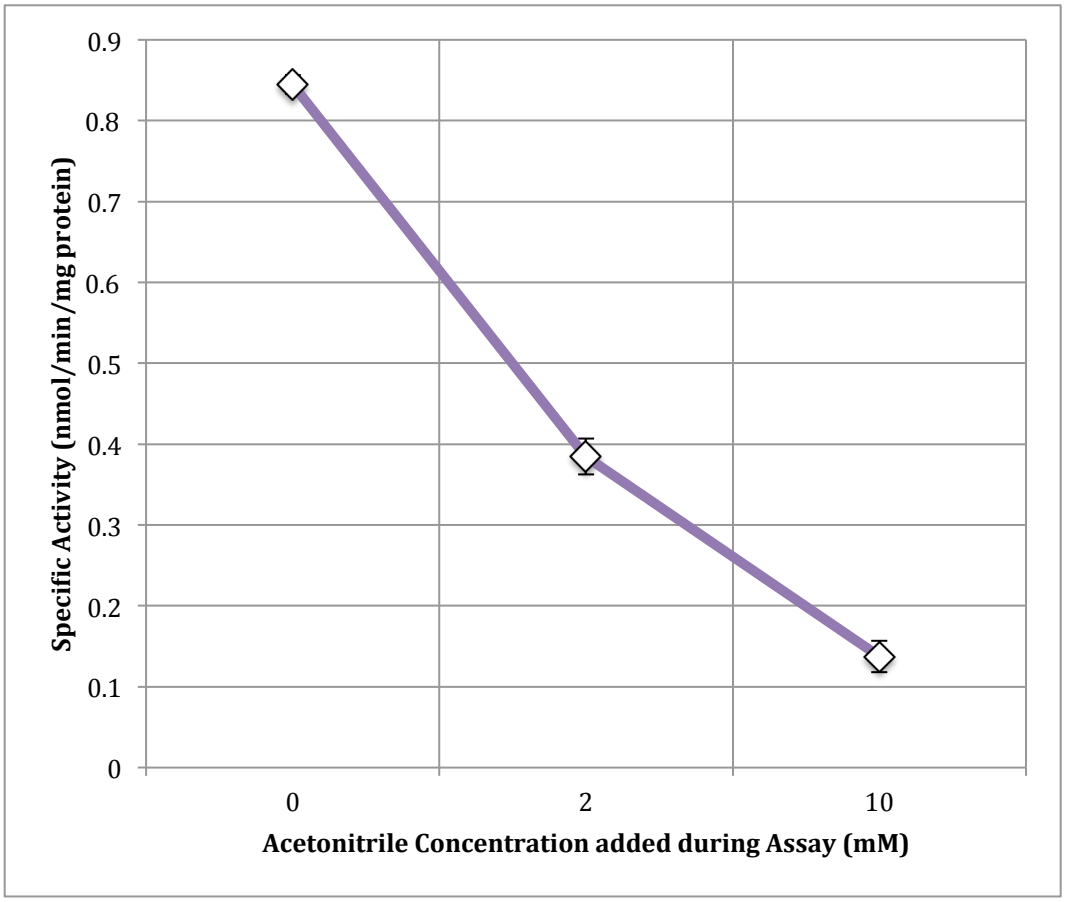
Table 23 and Figure 22 show the effects of adding acetonitrile to glucose thymine with 2 mM acetonitrile minimal media. Table 23 shows that a 2-fold increase in acetonitrile concentration resulted in a 2.2-fold decrease in specific activity and a 10-fold increase in acetonitrile concentration resulted in a 6.2-fold decrease in specific activity. Adding tryptophan to glucose thymine with 2 mM acetonitrile minimal media can be seen in Table 24 and Figure 23. It can be seen in Table 24 that a 5-fold increase in tryptophan concentration resulted in a 1-fold decrease in specific activity when adding tryptophan concentrations, a 1.3-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 2-fold decrease in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 23. Glucose Thymine with 2 mM Acetonitrile Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.845 ± 0.0119
2	0.385 ± 0.0224
10	0.137 ± 0.0195

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 22. *P. aeruginosa* grown in Glucose Thymine with 2 mM Acetonitrile Minimal Media adding Acetonitrile



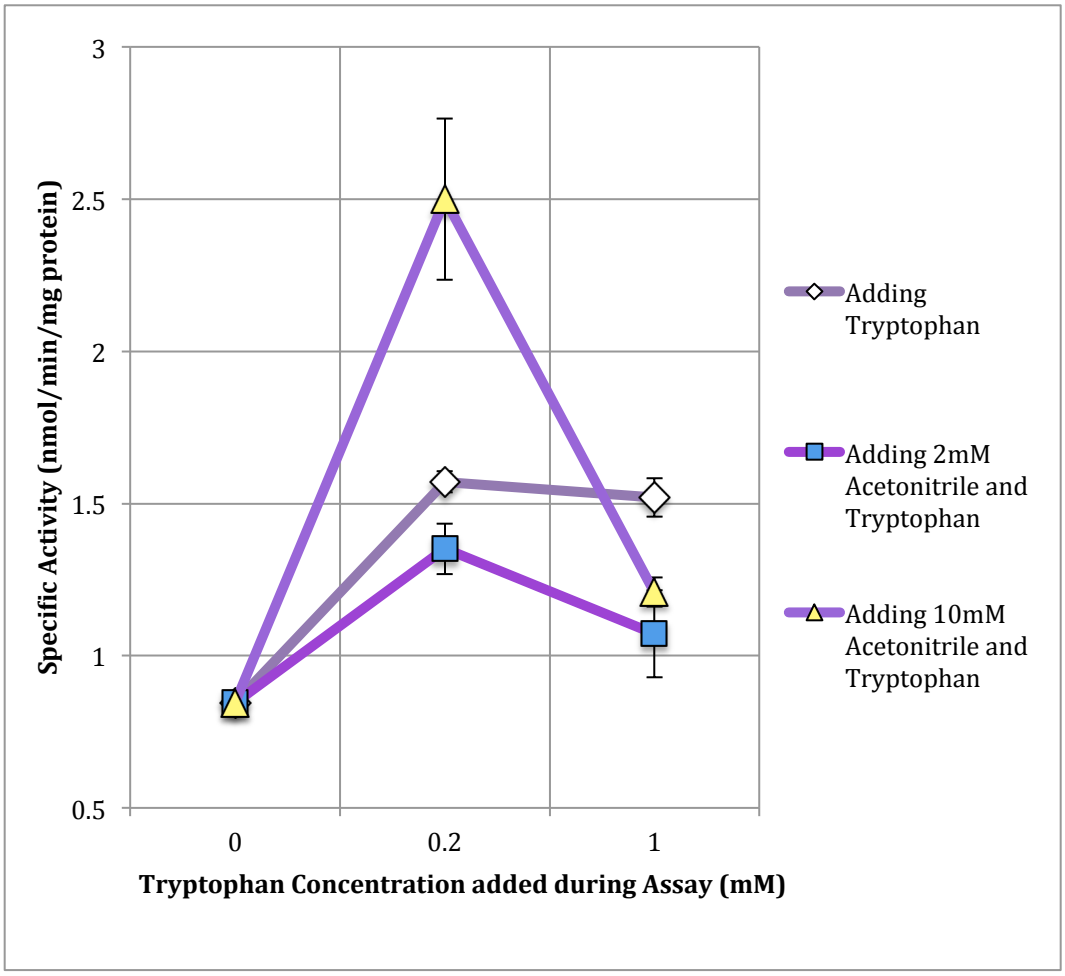
These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% glucose as the carbon source, and 2 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 24. Glucose Thymine with 2 mM Acetonitrile Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	1.57 ± 0.0348	1.35 ± 0.0828	2.50 ± 0.265
1.0	1.52 ± 0.0635	1.07 ± 0.143	1.21 ± 0.0476

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 23. *P. aeruginosa* grown in Glucose Thymine with 2 mM Acetonitrile Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% glucose as the carbon source, and 2 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

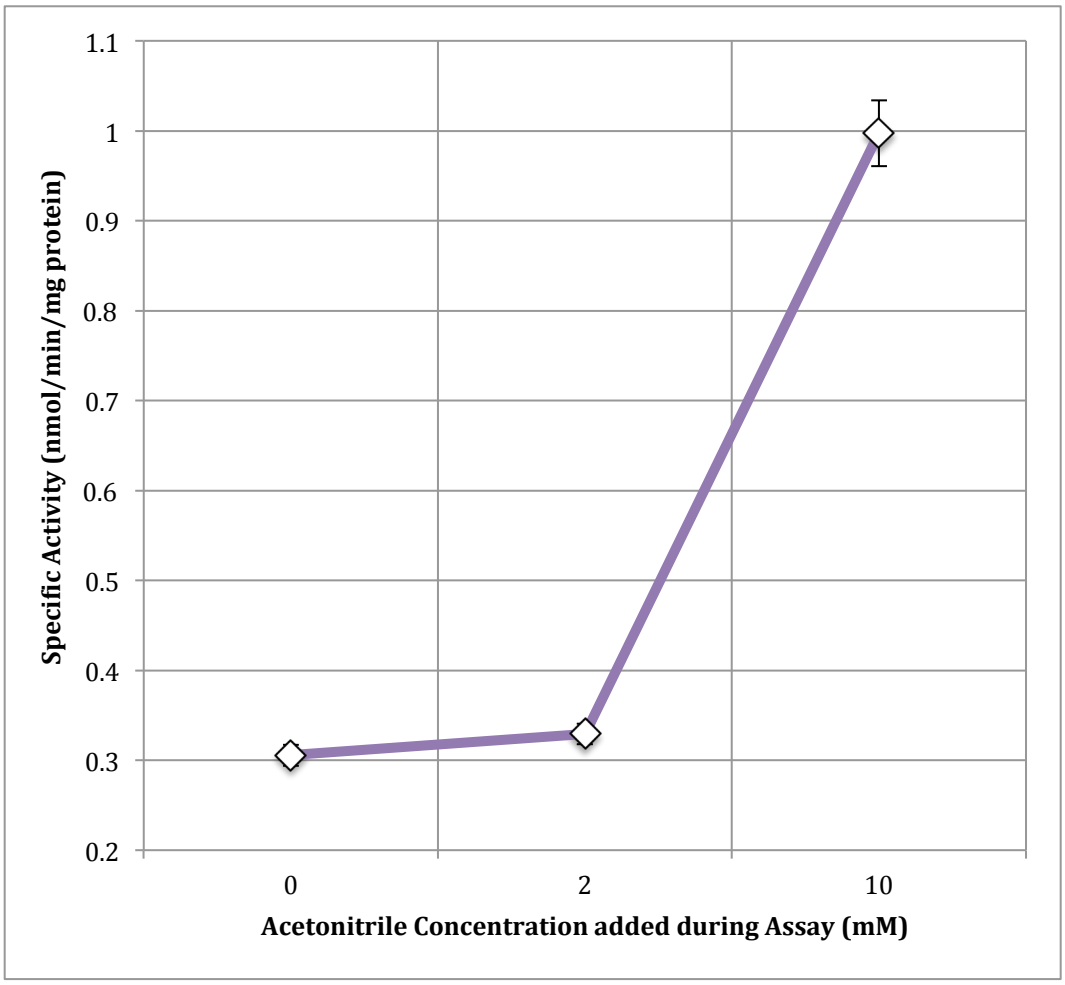
The effects of adding acetonitrile to glucose thymine with 10 mM acetonitrile minimal media can be seen in Table 25 and Figure 24. Table 25 shows that a 2-fold increase in acetonitrile concentration resulted in a 1.1-fold increase in specific activity and a 10-fold increase in acetonitrile concentration resulted in a 3.3-fold increase in specific activity. Table 26 and Figure 25 show the effects of adding tryptophan to glucose thymine with 10 mM acetonitrile minimal media. It can be seen in Table 26 that a 5-fold increase in tryptophan concentration resulted in a 1.4-fold decrease in specific activity when adding tryptophan concentrations, a 1.6-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.9-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 25. Glucose Thymine with 10 mM Acetonitrile Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.306 ± 0.0116
2	0.329 ± 0.0114
10	0.998 ± 0.0367

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 24. *P. aeruginosa* grown in Glucose Thymine with 10 mM Acetonitrile Minimal Media adding Acetonitrile



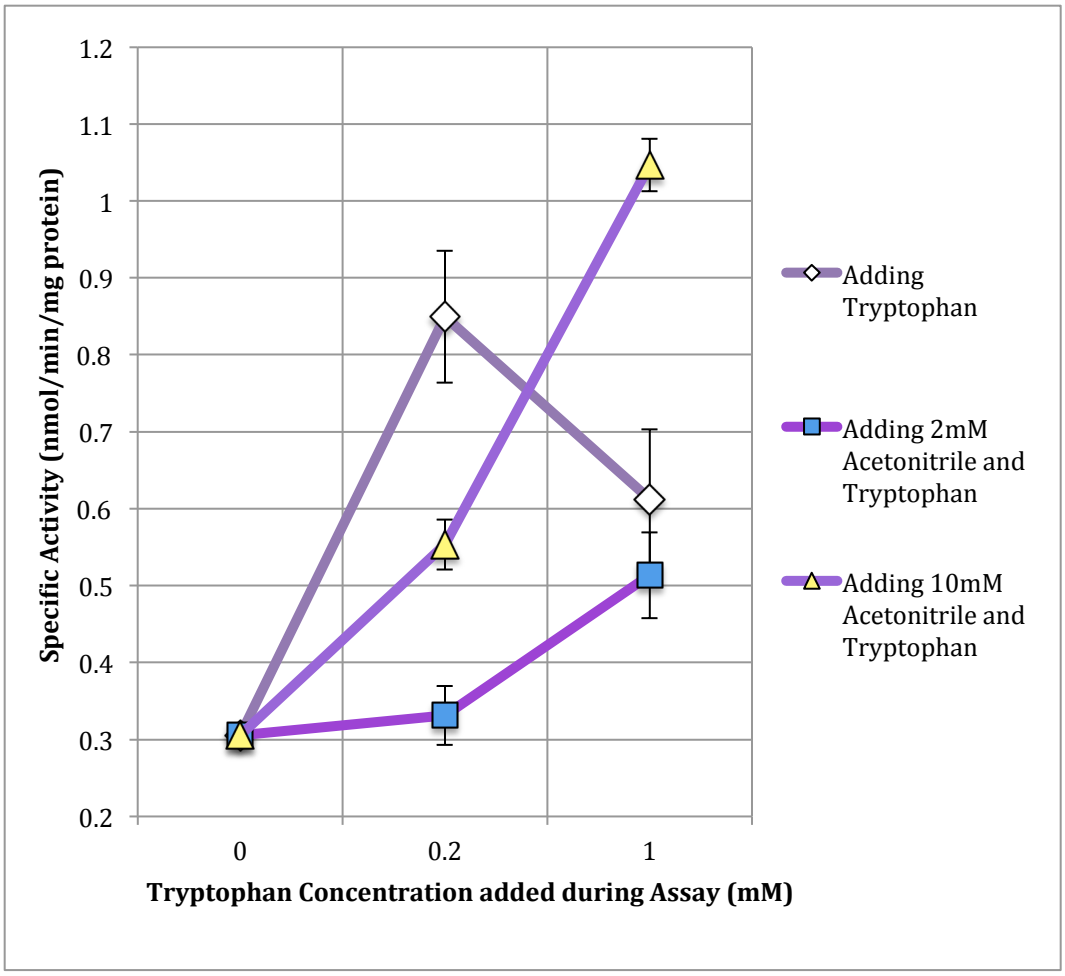
These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% glucose as the carbon source, and 10 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 26. Glucose Thymine with 10 mM Acetonitrile Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	0.849 ± 0.0856	0.331 ± 0.0383	0.553 ± 0.0323
1.0	0.612 ± 0.0905	0.514 ± 0.0559	1.05 ± 0.0342

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 25. *P. aeruginosa* grown in Glucose Thymine with 10 mM Acetonitrile Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% glucose as the carbon source, and 10 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

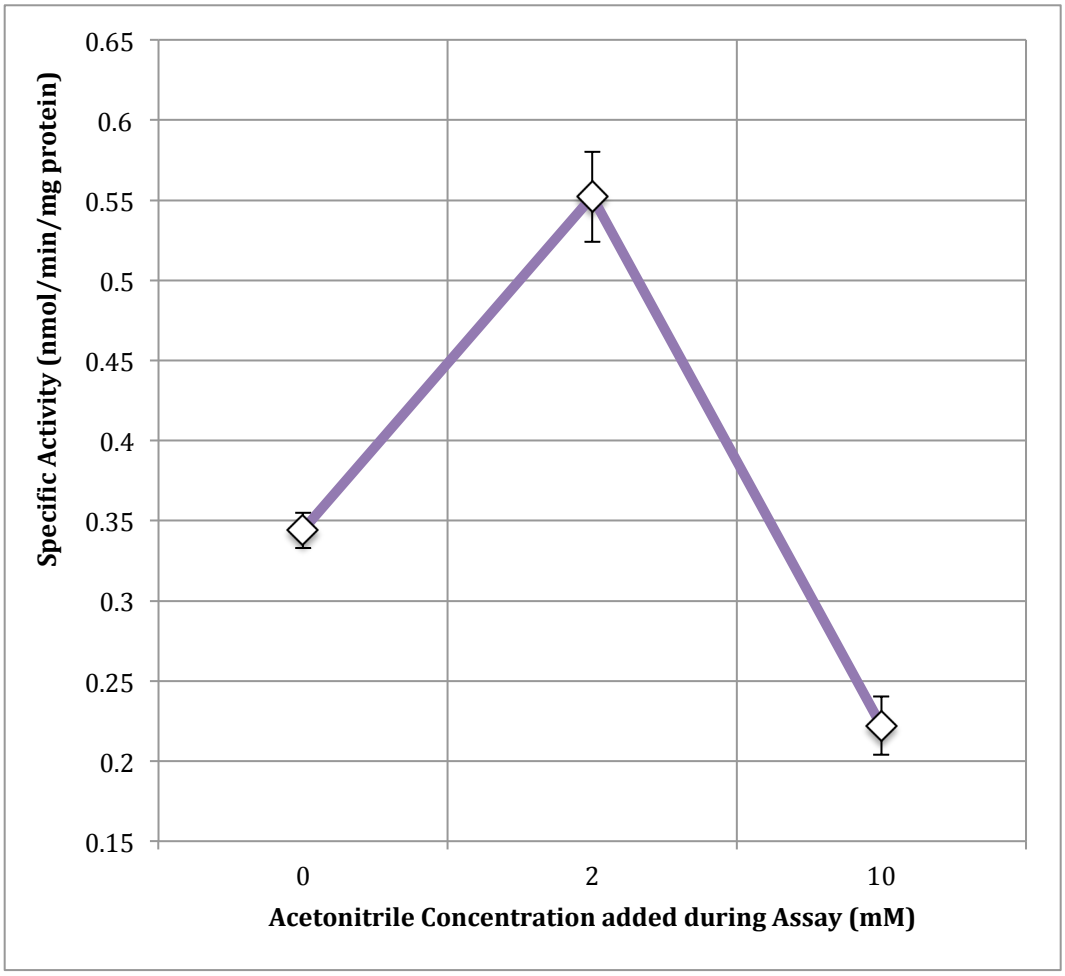
The adding of acetonitrile to succinate thymine with 2 mM acetonitrile minimal media can be seen in Table 27 and Figure 26. In Table 27, it can be seen that a 2-fold increase in acetonitrile concentration resulted in a 1.6-fold increase in specific activity and a 10-fold increase in acetonitrile concentration resulted in a 1.5-fold decrease in specific activity. Table 28 and Figure 27 show the effects of adding tryptophan to succinate thymine with 2 mM acetonitrile minimal media. It can be seen in Table 28 that a 5-fold increase in tryptophan concentration resulted in a 1-fold decrease in specific activity when adding tryptophan concentrations, a 1.5-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.1-fold decrease in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 27. Succinate Thymine with 2 mM Acetonitrile Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.344 ± 0.0111
2	0.552 ± 0.0281
10	0.222 ± 0.0181

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 26. *P. aeruginosa* grown in Succinate Thymine with 2 mM Acetonitrile Minimal Media adding Acetonitrile



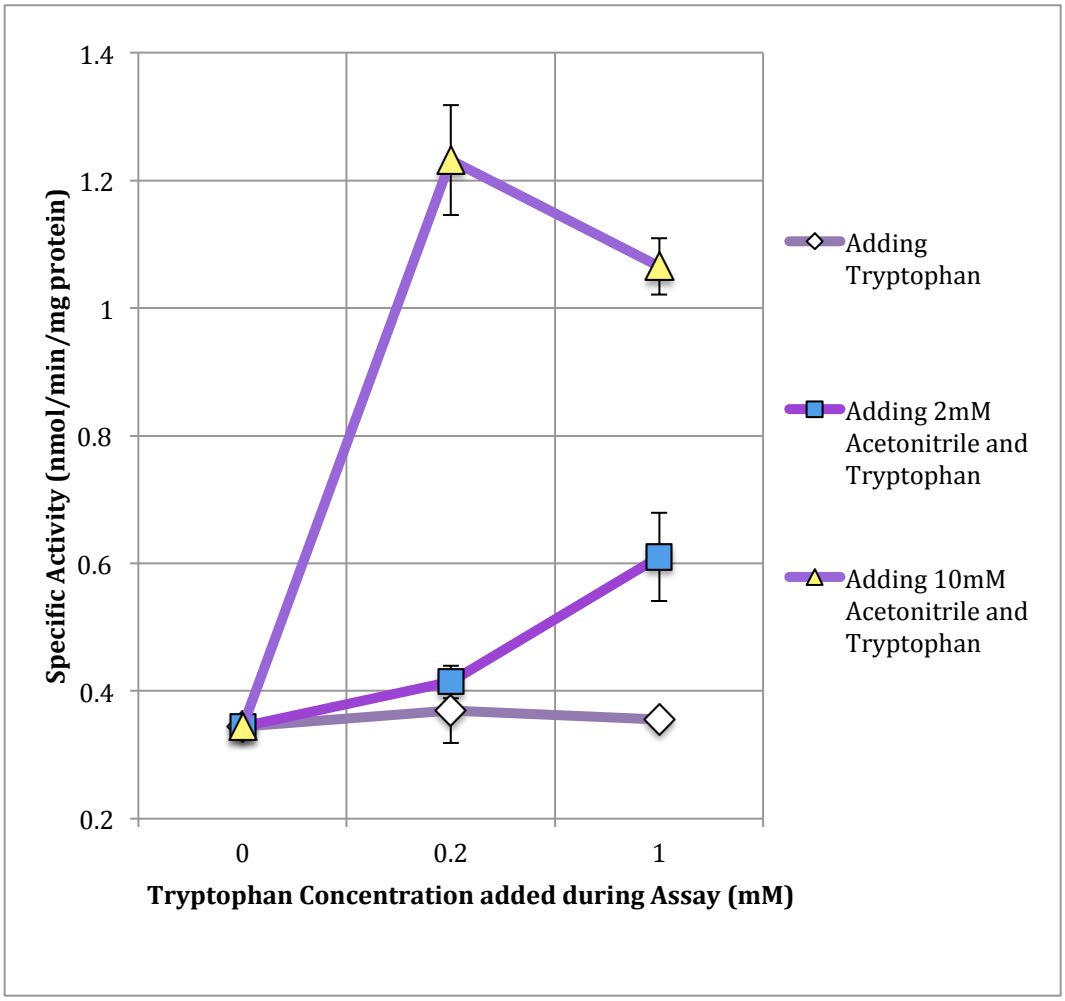
These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% succinate as the carbon source, and 2 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 28. Succinate Thymine with 2 mM Acetonitrile Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	0.370 ± 0.0513	0.414 ± 0.0256	1.23 ± 0.0860
1.0	0.355 ± 0.000	0.610 ± 0.0693	1.07 ± 0.0444

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 27. *P. aeruginosa* grown in Succinate Thymine with 2 mM Acetonitrile Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% succinate as the carbon source, and 2 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

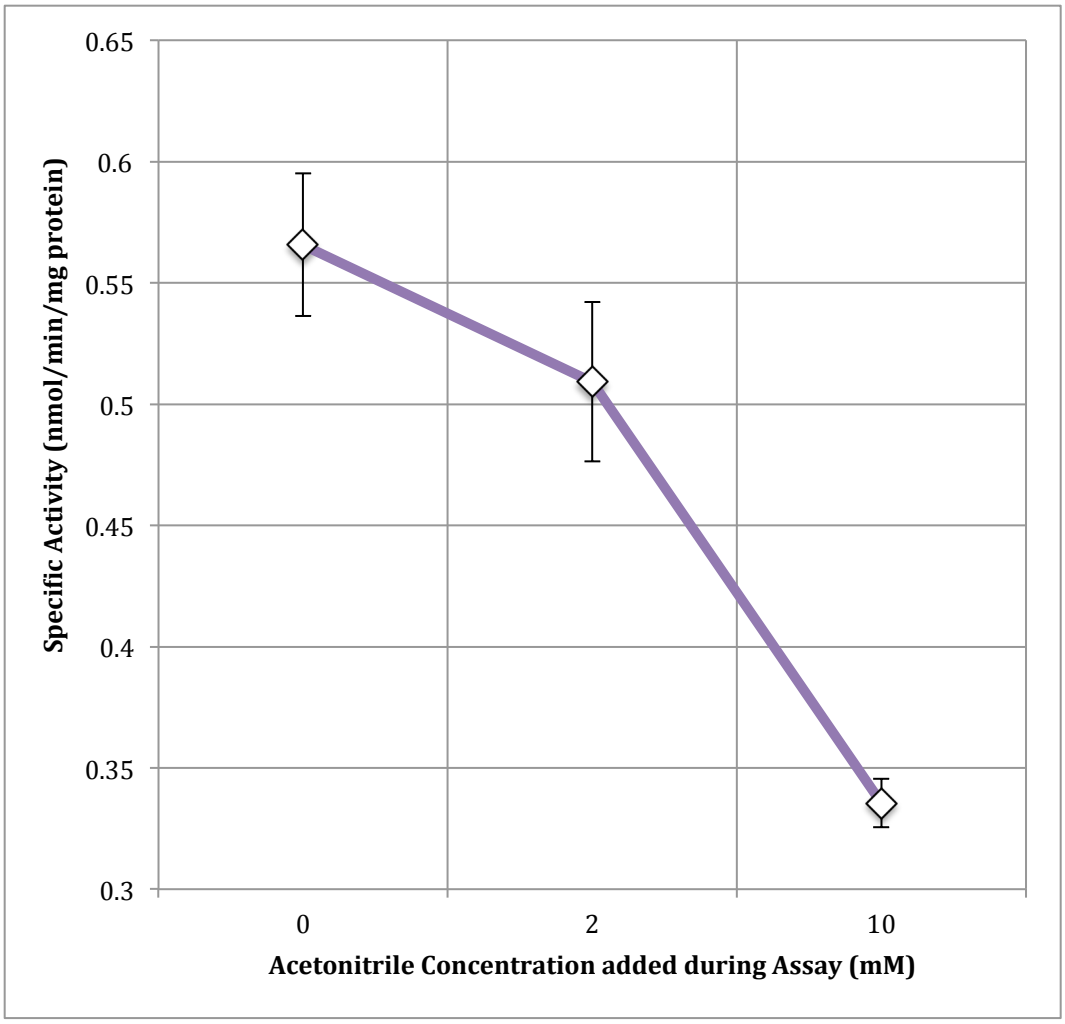
The effects of adding acetonitrile to succinate thymine with 10 mM acetonitrile minimal media can be seen in Table 29 and Figure 28. Table 29 shows that a 2-fold increase in acetonitrile concentration resulted in a 1.1-fold decrease in specific activity and a 10-fold increase in acetonitrile concentration resulted in a 1.7-fold decrease in specific activity. The adding of tryptophan to succinate thymine with 10 mM acetonitrile minimal media effects can be seen in Table 30 and Figure 29. It can be seen in Table 30 that a 5-fold increase in tryptophan concentration resulted in a 1.1-fold decrease in specific activity when adding tryptophan concentrations, a 1.3-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.8-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 29. Succinate Thymine with 10 mM Acetonitrile Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.566 ± 0.0294
2	0.509 ± 0.0329
10	0.336 ± 0.00994

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 28. *P. aeruginosa* grown in Succinate Thymine with 10 mM Acetonitrile Minimal Media adding Acetonitrile



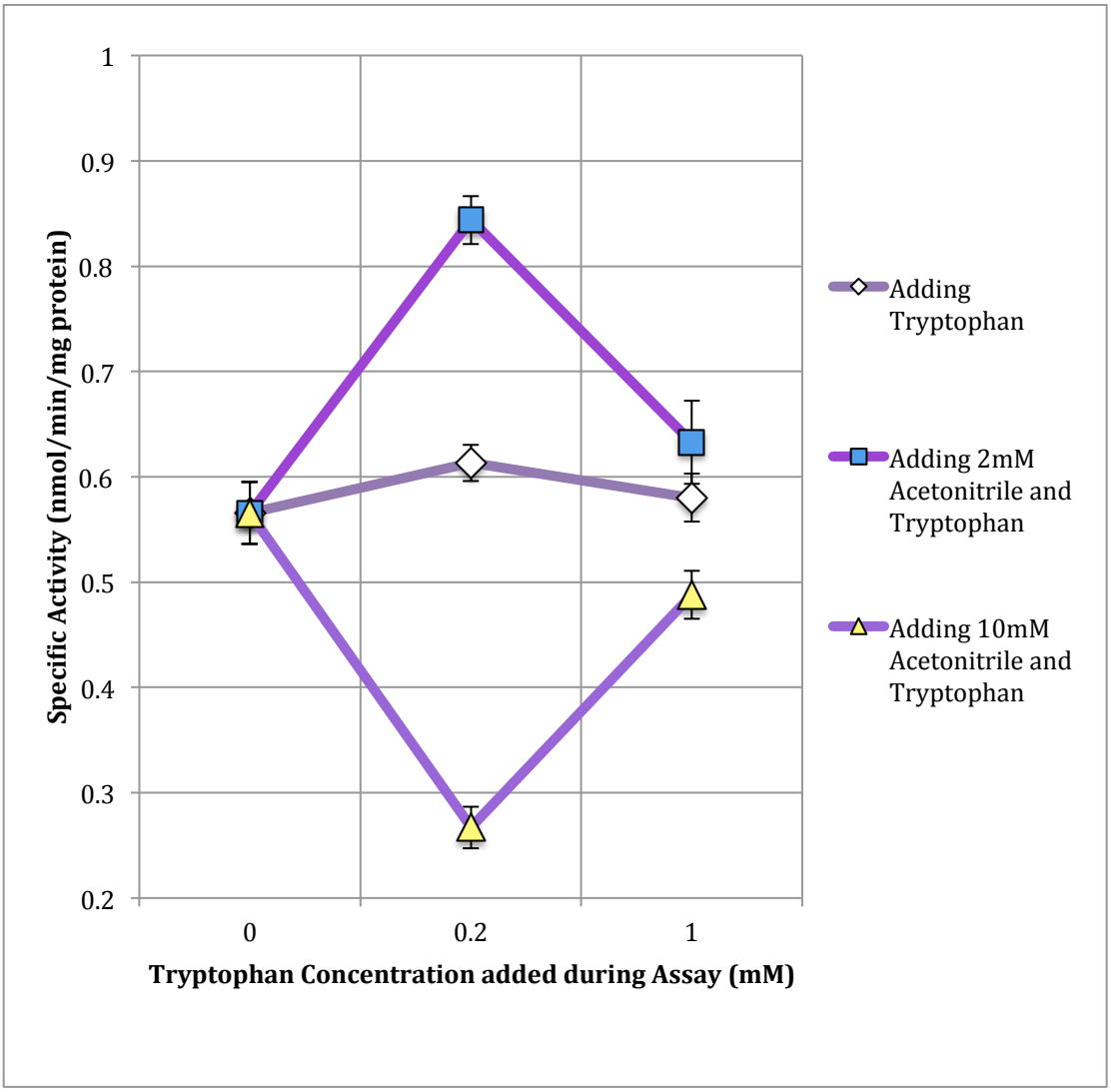
These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% succinate as the carbon source, and 10 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 30. Succinate Thymine with 10 mM Acetonitrile Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0.2	0.613 ± 0.0172	0.844 ± 0.0229	0.267 ± 0.0198
1.0	0.580 ± 0.0228	0.633 ± 0.0396	0.488 ± 0.0229

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 29. *P. aeruginosa* grown in Succinate Thymine with 10 mM Acetonitrile Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% succinate as the carbon source, and 10 mM acetonitrile. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

When the cells were grown in minimal media containing glucose or succinate as the carbon source, uracil or thymine as the nitrogen source and 0.2 mM or 1 mM concentrations of tryptophan generation times were found (Table 31 and Table 32). It can be seen with these minimal media that the generation times varied dependent upon the nitrogen source and the concentration of tryptophan. When glucose was the carbon source with 0.2 mM tryptophan, the change from uracil to thymine as the nitrogen source resulted in a 9.6 fold increase in the generation time. Whereas, when succinate was the carbon source with 0.2 mM tryptophan, the change from uracil to thymine as the nitrogen source resulted in a 2.49-fold decrease in generation time. Having 1 mM tryptophan with glucose, as the carbon source, and changing the nitrogen source from uracil to thymine resulted in a 2-fold increase in generation time. When succinate was the carbon source with 1 mM tryptophan, the change from uracil to thymine as the nitrogen source resulted in a 3.8-fold increase in the generation time.

Table 31. Generation Times in Glucose or Succinate and Uracil or Thymine with 0.2 mM Tryptophan

Glucose Uracil with 0.2mM Tryptophan (minutes)	Glucose Thymine with 0.2mM Tryptophan (minutes)	Succinate Uracil with 0.2mM Tryptophan (minutes)	Succinate Thymine with 0.2mM Tryptophan (minutes)
91 ± 8	877 ± 30	249 ± 23	100 ± 37

Table 32. Generation Times in Glucose or Succinate and Uracil or Thymine with 1 mM Tryptophan

Glucose Uracil with 1mM Tryptophan (minutes)	Glucose Thymine with 1mM Tryptophan (minutes)	Succinate Uracil with 1mM Tryptophan (minutes)	Succinate Thymine with 1mM Tryptophan (minutes)
166 ± 15	331 ± 91	92 ± 15	347 ± 46

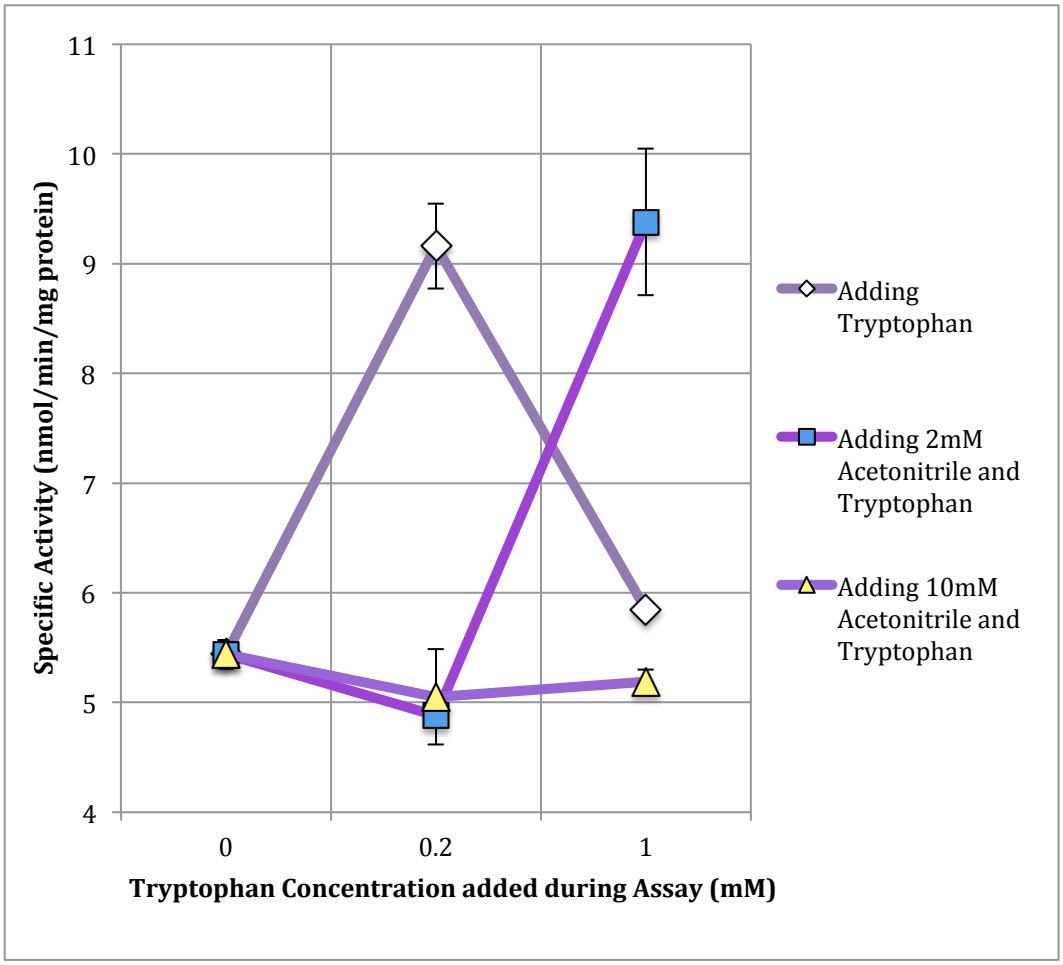
In order to determine the effects of the addition of acetonitrile, tryptophan and ammonia the dihydropyrimidine dehydrogenase (DHPD) assay was performed. This assay was performed on each of the cell-free extracts in Tables 31 and 32.The effects of adding tryptophan to Glucose Uracil with 0.2 mM Tryptophan can be seen in Table 33 and Figure 30. It can be seen in Table 33 that a 1-fold increase in tryptophan concentration resulted in a 1.1-fold increase in specific activity when adding tryptophan concentrations, a 1.7-fold increase when adding 2 mM acetonitrile and tryptophan concentrations, and a 1-fold decrease when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 33 that a 5-fold increase in tryptophan concentration resulted in a 1.6-fold decrease in specific activity when adding tryptophan concentrations, a 1.9-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 33. Glucose Uracil with 0.2 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	5.44 ± 0.129	5.44 ± 0.129	5.44 ± 0.129
0.2	9.16 ± 0.387	4.88 ± 0.0322	5.05 ± 0.434
1.0	5.85 ± 0.0323	9.38 ± 0.670	5.19 ± 0.112

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 30. *P. aeruginosa* grown in Glucose Uracil with 0.2 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, and 0.2 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

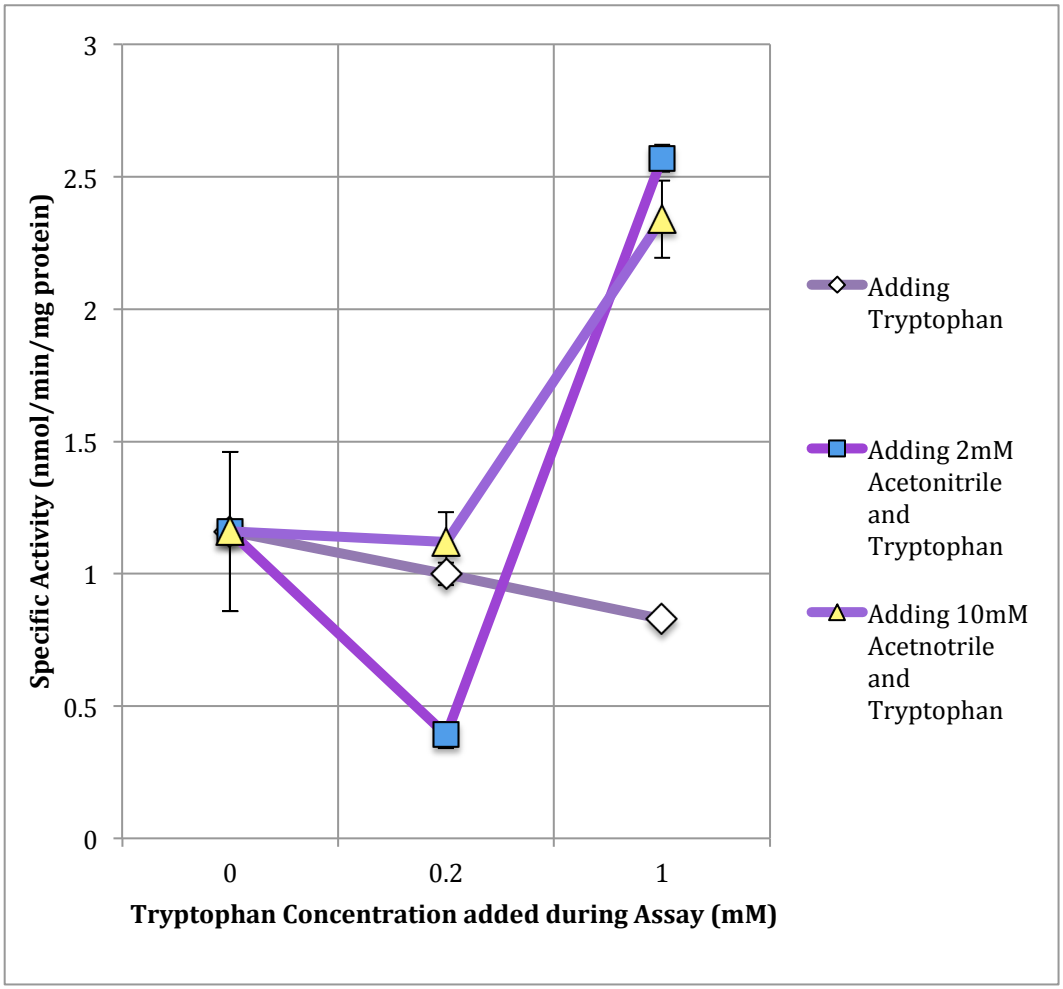
Table 34 and Figure 31 show the effects of adding tryptophan to glucose uracil with 1 mM tryptophan minimal media. It can be seen in Table 34 that a 1-fold increase in tryptophan concentration resulted in a 1.4-fold decrease in specific activity when adding tryptophan concentrations, a 2.2-fold increase when adding 2 mM acetonitrile and tryptophan concentrations, and a 2-fold increase when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 34 that a 5-fold increase in tryptophan concentration resulted in a 1.2-fold decrease in specific activity when adding tryptophan concentrations, a 6.6-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 2.1-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 34. Glucose Uracil with 1 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg portein)
0	1.16 ± 0.301	1.16 ± 0.301	1.16 ± 0.301
0.2	0.999 ± 0.0422	0.390 ± 0.0488	1.12 ± 0.112
1.0	0.828 ± 0.0211	2.57 ± 0.0507	2.34 ± 0.146

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 31. *P. aeruginosa* grown in Glucose Uracil with 1 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, and 1 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

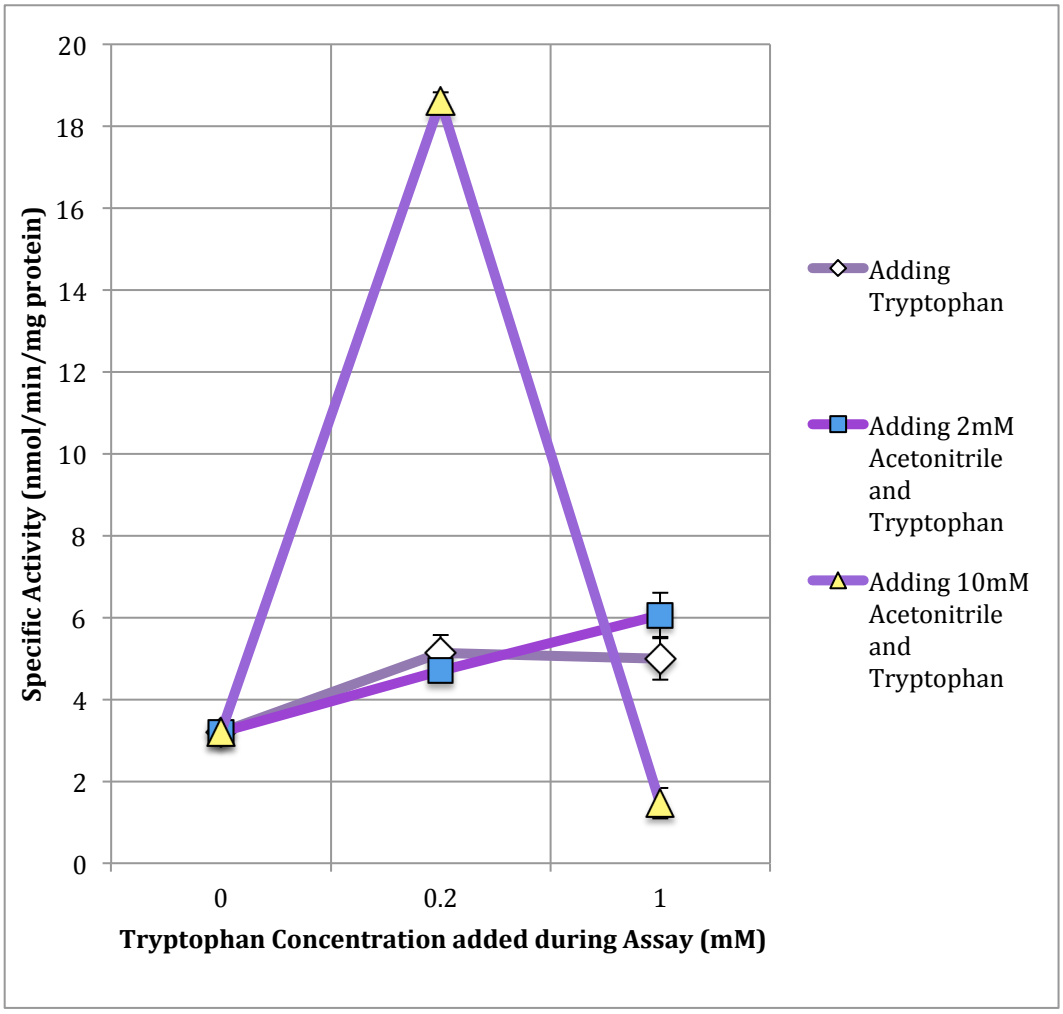
Adding tryptophan to succinate uracil with 0.2 mM tryptophan minimal media effects can be seen in Table 35 and Figure 32. It can be seen in Table 35 that a 1-fold increase in tryptophan concentration resulted in a 1.6-fold increase in specific activity when adding tryptophan concentrations, a 1.9-fold increase when adding 2 mM acetonitrile and tryptophan concentrations, and a 2.2-fold decrease when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 35 that a 5-fold increase in tryptophan concentration resulted in a 1-fold decrease in specific activity when adding tryptophan concentrations, a 1.3-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 12.7-fold decrease in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 35. Succinate Uracil with 0.2 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	3.20 ± 0.226	3.20 ± 0.226	3.20 ± 0.226
0.2	5.13 ± 0.440	4.69 ± 0.000	18.6 ± 0.226
1.0	4.99 ± 0.508	6.06 ± 0.536	1.47 ± 0.375

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 32. *P. aeruginosa* grown in Succinate Uracil with 0.2 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% succinate as the carbon source, and 0.2 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

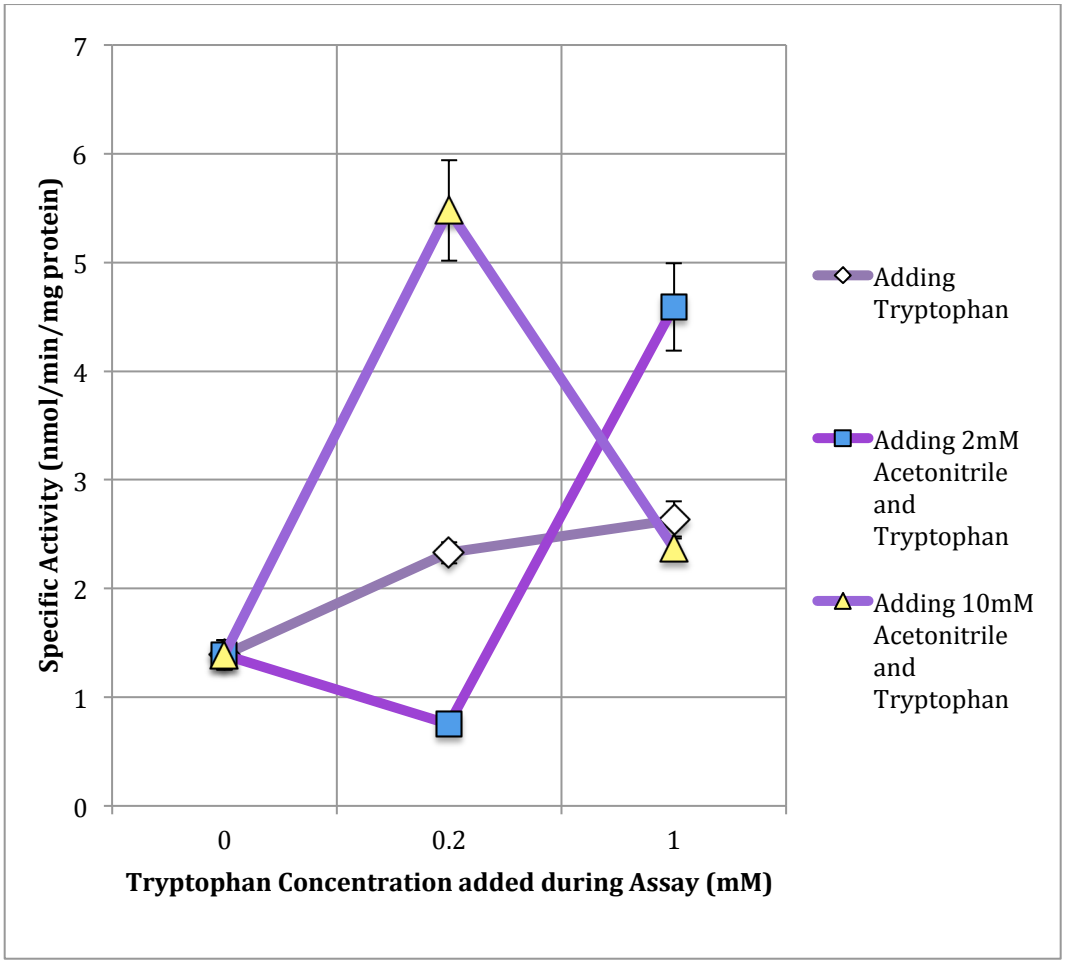
The effects of adding tryptophan to succinate uracil with 1 mM tryptophan can be seen in Table 36 and Figure 33. It can be seen in Table 36 that a 1-fold increase in tryptophan concentration resulted in a 1.9-fold increase in specific activity when adding tryptophan concentrations, a 3.3-fold increase when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.7-fold increase when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 36 that a 5-fold increase in tryptophan concentration resulted in a 1.1-fold increase in specific activity when adding tryptophan concentrations, a 6.1-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 2.3-fold decrease in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 36. Succinate Uracil with 1 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	1.39 ± 0.134	1.39 ± 0.134	1.39 ± 0.134
0.2	2.33 ± 0.0981	0.751 ± 0.0805	5.48 ± 0.464
1.0	2.63 ± 0.170	4.59 ± 0.401	2.37 ± 0.111

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 33. *P. aeruginosa* grown in Succinate Uracil with 1 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% succinate as the carbon source, and 1 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

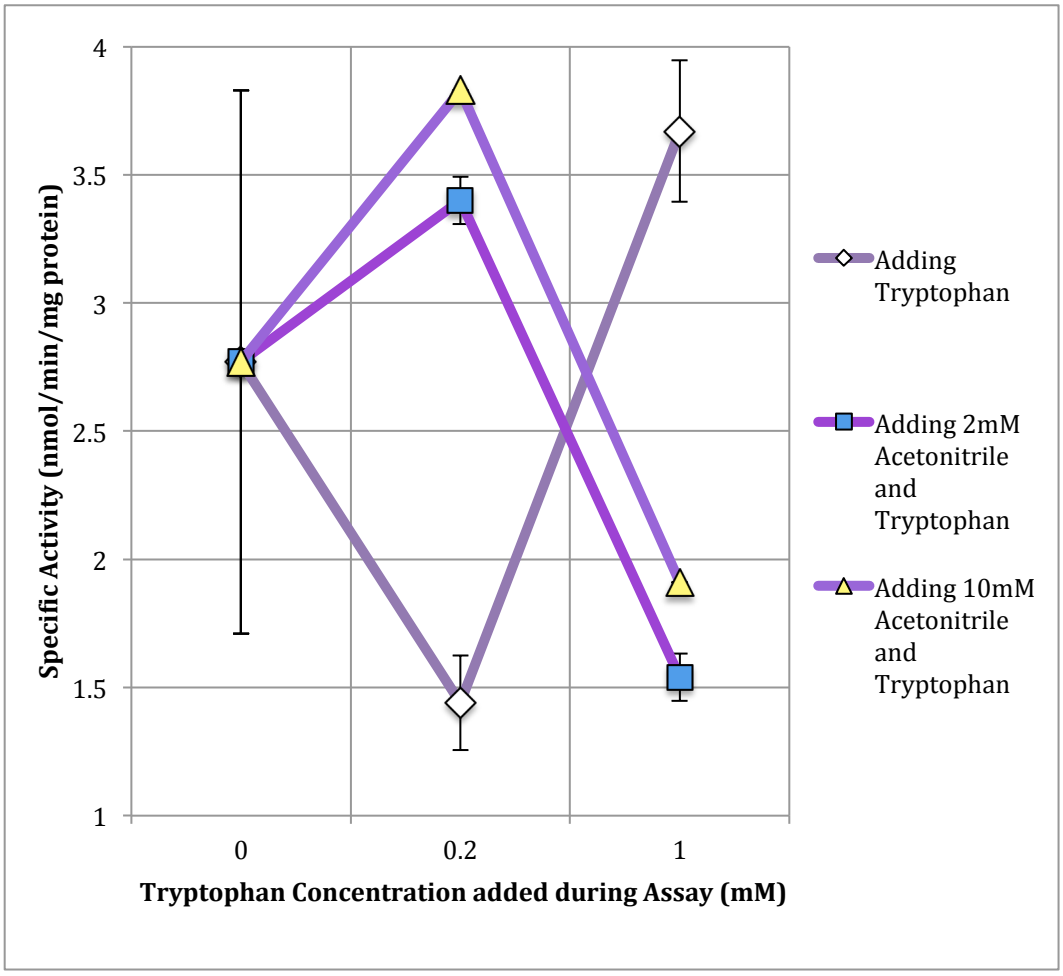
Table 37 and Figure 34 show the effects of adding tryptophan to glucose thymine with 0.2 mM tryptophan minimal media. It can be seen in Table 37 that a 1-fold increase in tryptophan concentration resulted in a 1.3-fold increase in specific activity when adding tryptophan concentrations, a 1.8-fold decrease when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.4-fold decrease when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 37 that a 5-fold increase in tryptophan concentration resulted in a 2.6-fold increase in specific activity when adding tryptophan concentrations, a 2.2-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 2-fold decrease in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 37. Glucose Thymine with 0.2 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	2.77 ± 1.06	2.77 ± 1.06	2.77 ± 1.06
0.2	1.44 ± 0.184	3.40 ± 0.0921	3.83 ± 0.000
1.0	3.67 ± 0.276	1.54 ± 0.0921	1.91 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 34. *P. aeruginosa* grown in Glucose Thymine with 0.2 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% glucose as the carbon source, and 0.2 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

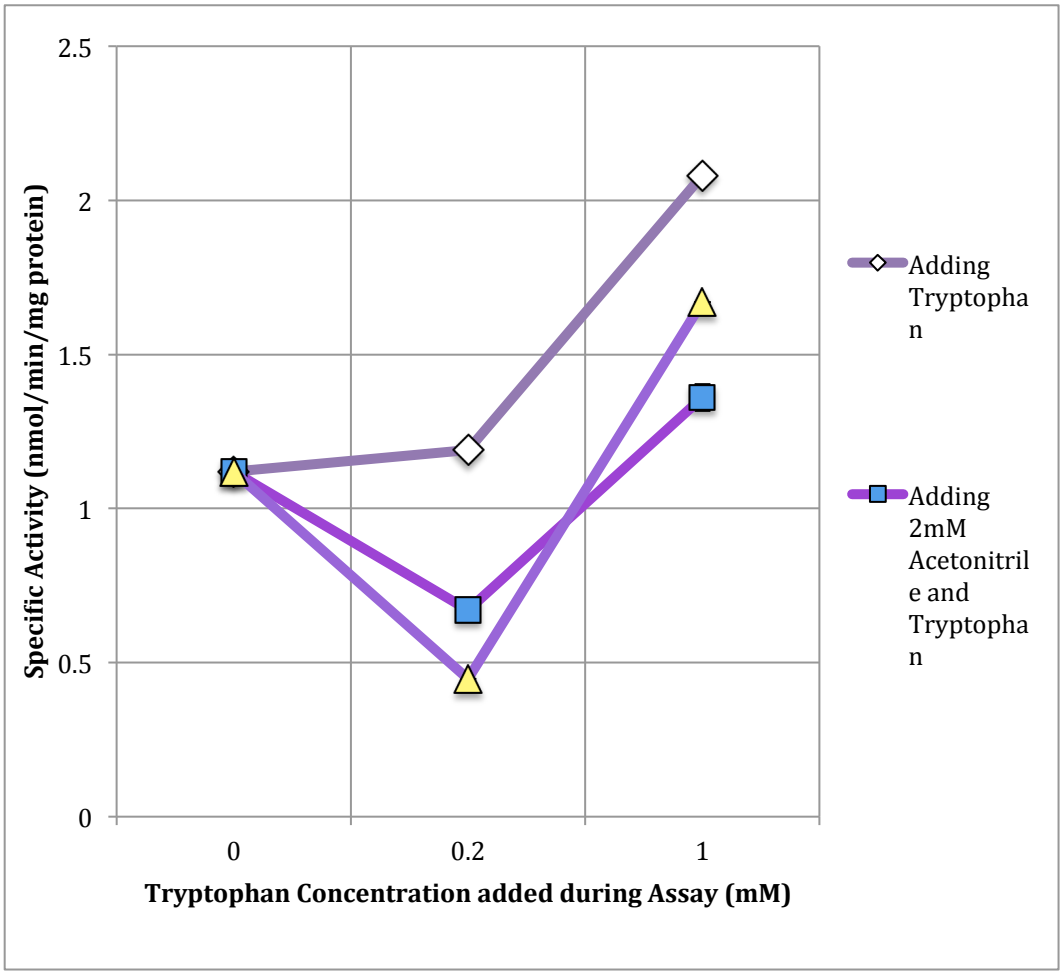
The effects of adding tryptophan to glucose thymine with 1 mM tryptophan can be seen in Table 38 and Figure 35. It can be seen in Table 38 that a 1-fold increase in tryptophan concentration resulted in a 1.9-fold increase in specific activity when adding tryptophan concentrations, a 1.2-fold increase when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.5-fold increase when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 38 that a 5-fold increase in tryptophan concentration resulted in a 1.8-fold increase in specific activity when adding tryptophan concentrations, a 2-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 3.7-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 38. Glucose Thymine with 1 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	1.12 ± 0.000	1.12 ± 0.000	1.12 ± 0.000
0.2	1.19 ± 0.000	0.670 ± 0.000	0.447 ± 0.000
1.0	2.08 ± 0.000	1.36 ± 0.0430	1.67 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 35. *P. aeruginosa* grown in Glucose Thymine with 1 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% glucose as the carbon source, and 1 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

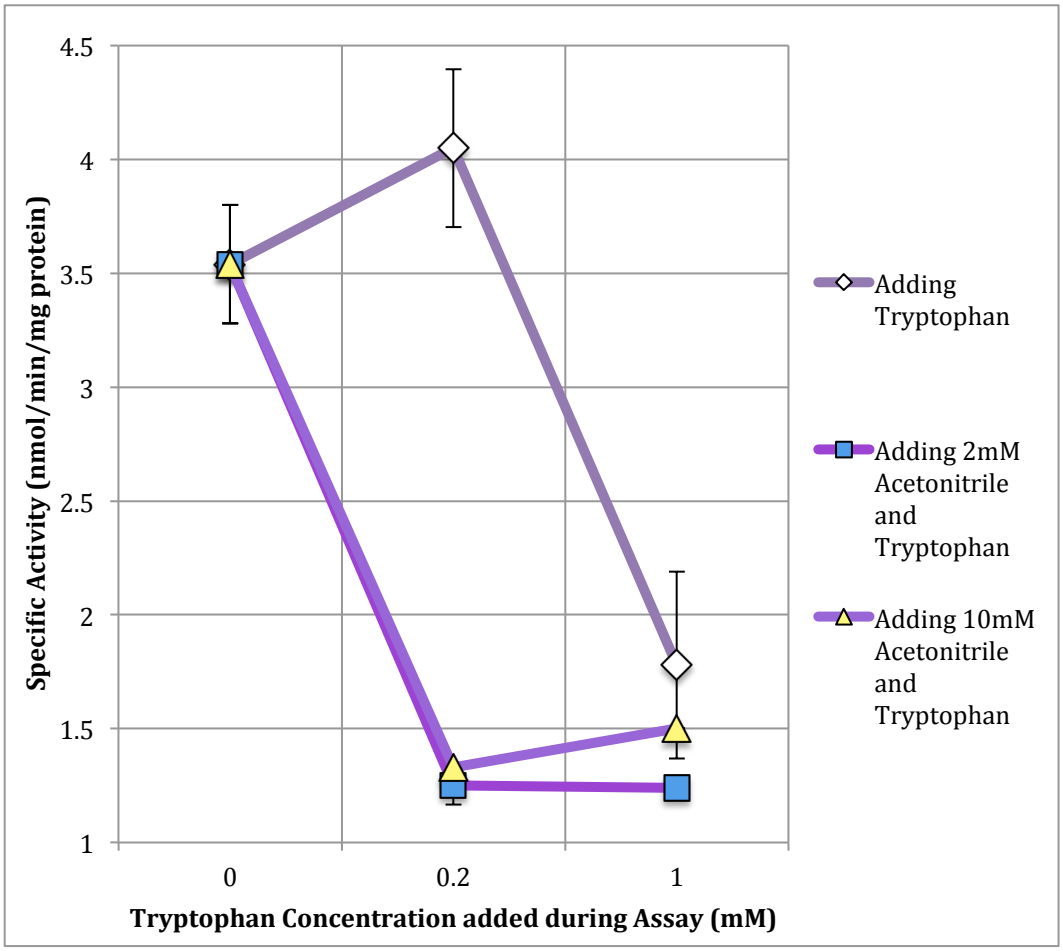
When adding tryptophan to succinate thymine with 0.2 mM tryptophan, the effects can be seen in Table 39 and Figure 36. It can be seen in Table 39 that a 1-fold increase in tryptophan concentration resulted in a 2-fold decrease in specific activity when adding tryptophan concentrations, a 2.9-fold decrease when adding 2 mM acetonitrile and tryptophan concentrations, and a 2.4-fold decrease when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 39 that a 5-fold increase in tryptophan concentration resulted in a 2.3-fold decrease in specific activity when adding tryptophan concentrations, a 1-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.1-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 39. Succinate Thymine with 0.2 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	3.54 ± 0.260	3.54 ± 0.260	3.54 ± 0.260
0.2	4.05 ± 0347	1.25 ± 0.0833	1.33 ± 0.000
1.0	1.78 ± 0.411	1.24± 0.0160	1.50 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 36. *P. aeruginosa* grown in Succinate Thymine with 0.2 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% succinate as the carbon source, and 0.2 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

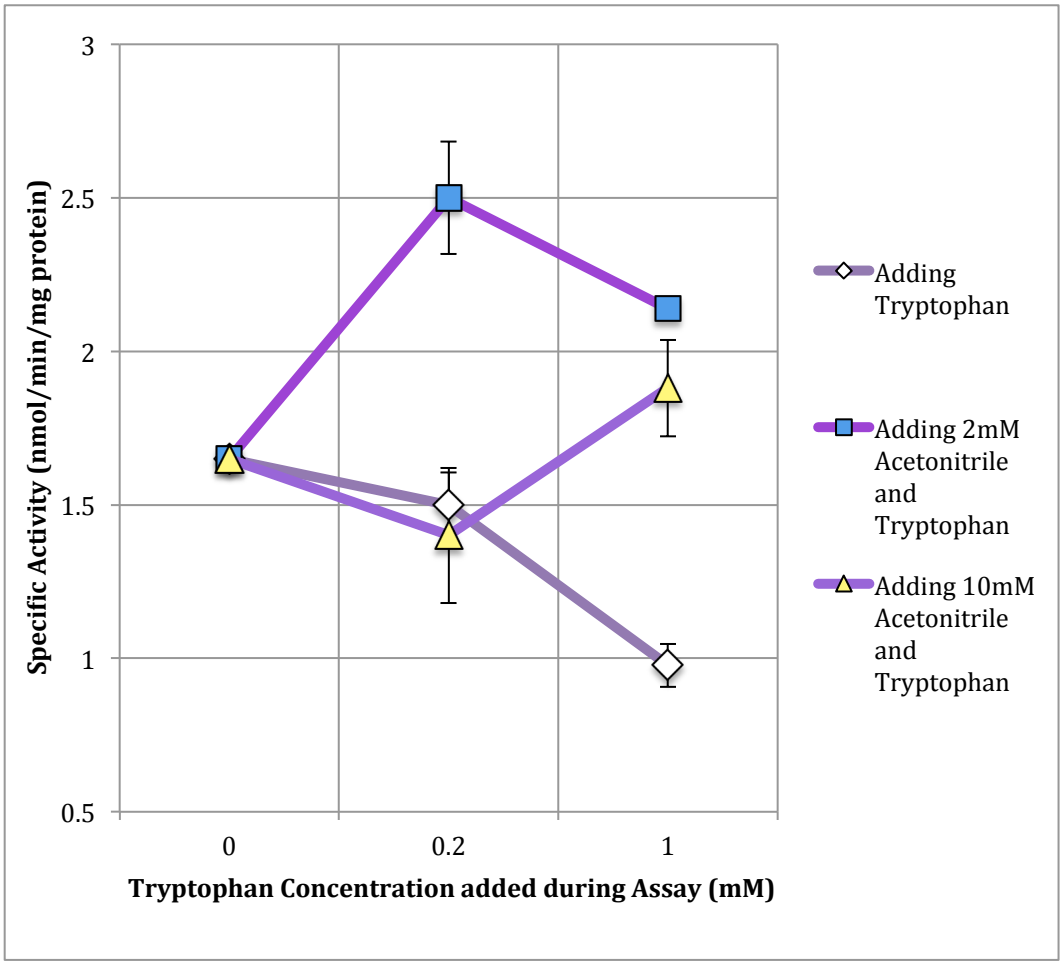
The effects of adding tryptophan to succinate thymine with 1 mM tryptophan can be seen in Table 40 and Figure 37. It can be seen in Table 40 that a 1-fold increase in tryptophan concentration resulted in a 1.7-fold decrease in specific activity when adding tryptophan concentrations, a 1.3-fold increase when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.1-fold increase when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 40 that a 5-fold increase in tryptophan concentration resulted in a 1.5-fold decrease in specific activity when adding tryptophan concentrations, a 1.2-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.3-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 40. Succinate Thymine with 1 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	1.65 ± 0.000	1.65 ± 0.000	1.65 ± 0.000
0.2	1.50 ± 0.106	2.50 ± 0.183	1.40 ± 0.220
1.0	0.977 ± 0.0704	2.14 ± 0.000	1.88 ± 0.157

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 37. *P. aeruginosa* grown in Succinate Thymine with 1mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% thymine as the source of nitrogen, 0.4% succinate as the carbon source, and 1 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

When the cells were grown in minimal media containing glucose as the carbon source, uracil as the nitrogen source, 0.2 mM or 1 mM concentrations of tryptophan, and 2 mM or 10 mM concentrations of acetonitrile generation times were found (Table 41). It can be seen with these minimal media that the generation times varied dependent upon the concentration of tryptophan and the concentration of acetonitrile. Glucose uracil with 2 mM acetonitrile and 0.2 mM tryptophan and glucose uracil with 10 mM acetonitrile and 1mM tryptophan had very similar generation times differing only by 1-fold. Glucose uracil with 2 mM acetonitrile and 1 mM tryptophan and glucose uracil with 10mM acetonitrile and 0.2 mM tryptophan had very similar generation times differing only by 1.2-fold. The two extremes, glucose uracil with 2 mM acetonitrile and 0.2 mM tryptophan and 10 mM acetonitrile and 1 mM tryptophan, have a 1.3-fold decrease in generation time from that of glucose uracil with 2 mM acetonitrile and 1 mM tryptophan and a 1.6-fold decrease in generation time from that of glucose uracil with 10 mM acetonitrile and 0.2 mM tryptophan.

Table 41. Generation Times in Glucose Uracil with 2 mM or 10 mM Acetonitrile and 0.2 mM or 1 mM Tryptophan

Glucose Uracil with 2mM Acetonitrile and 0.2mM Tryptophan (minutes)	Glucose Uracil with 2mM Acetonitrile and 1mM Tryptophan (minutes)	Glucose Uracil with 10mM Acetonitrile and 0.2mM Tryptophan (minutes)	Glucose Uracil with 10mM Acetonitrile and 1mM Tryptophan (minutes)
255 ± 13	328 ± 42	408 ± 26	257 ± 56

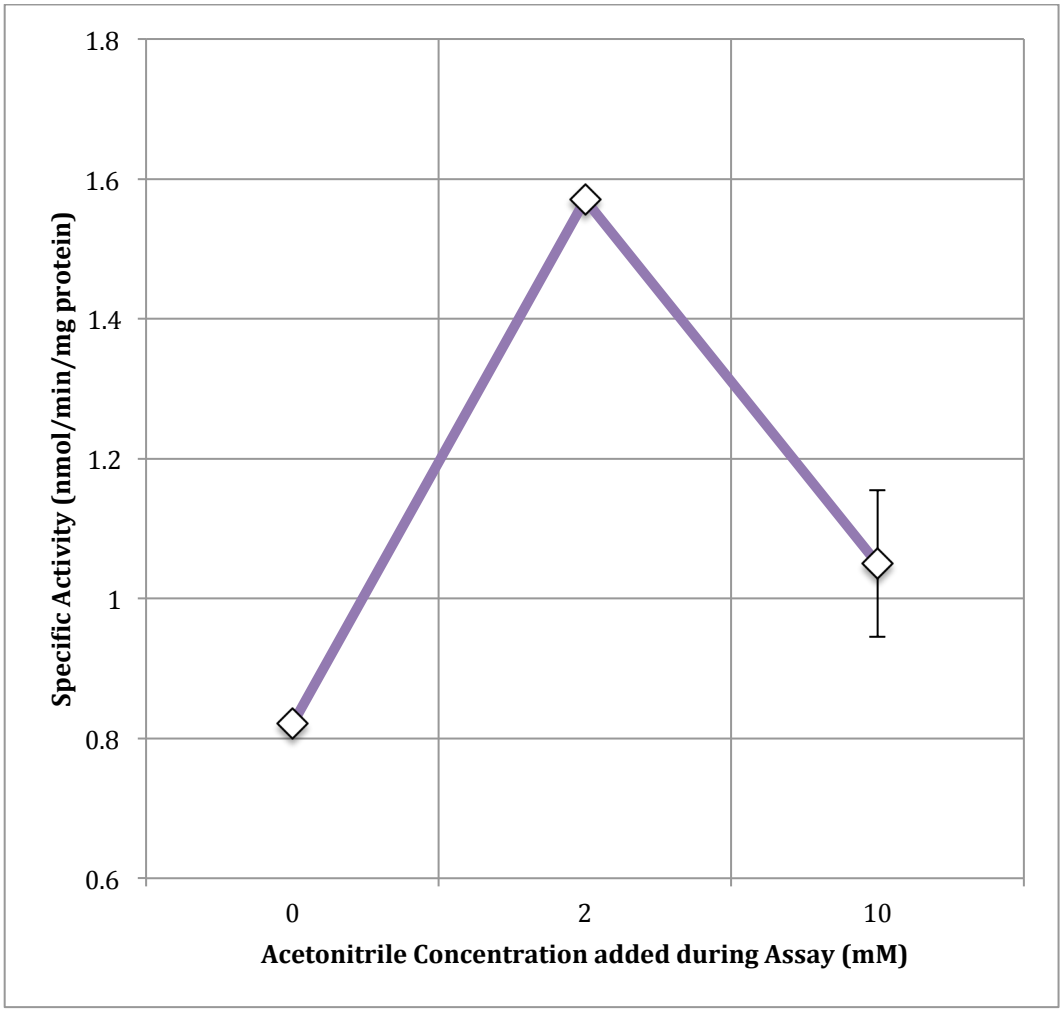
In order to determine the effects of the addition of acetonitrile, tryptophan and ammonia the dihydropyrimidine dehydrogenase (DHPD) assay was performed. This assay was performed on each of the cell-free extracts in Table 41. The effects of adding acetonitrile to glucose uracil with 2 mM acetonitrile and 0.2 mM tryptophan minimal media can be seen in Table 42 and Figure 38. It can be seen in Table 42 that 2-fold increase in acetonitrile concentration resulted in a 1.9-fold increase in specific activity and a 10-fold increase in acetonitrile concentration resulted in a 1.3-fold increase in specific activity. Table 43 and Figure 39 show the effects of adding tryptophan to glucose uracil with 2 mM acetonitrile and 0.2 mM tryptophan minimal media. It can be seen in Table 43 that a 1-fold increase in tryptophan resulted in a 2.4-fold increase in specific activity when adding tryptophan concentrations, a 2.2-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.6-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 43 that a 5-fold increase in tryptophan concentration resulted in a 1.4-fold decrease in specific activity when adding tryptophan concentrations, a 2.2-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and no change in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 42. Glucose Uracil with 2 mM Acetonitrile and 0.2 mM Tryptophan Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.821 ± 0.000
2	1.57 ± 0.000
10	1.05 ± 0.105

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 38. *P. aeruginosa* grown in Glucose Uracil with 2 mM Acetonitrile and 0.2 mM Tryptophan Minimal Media adding Acetonitrile



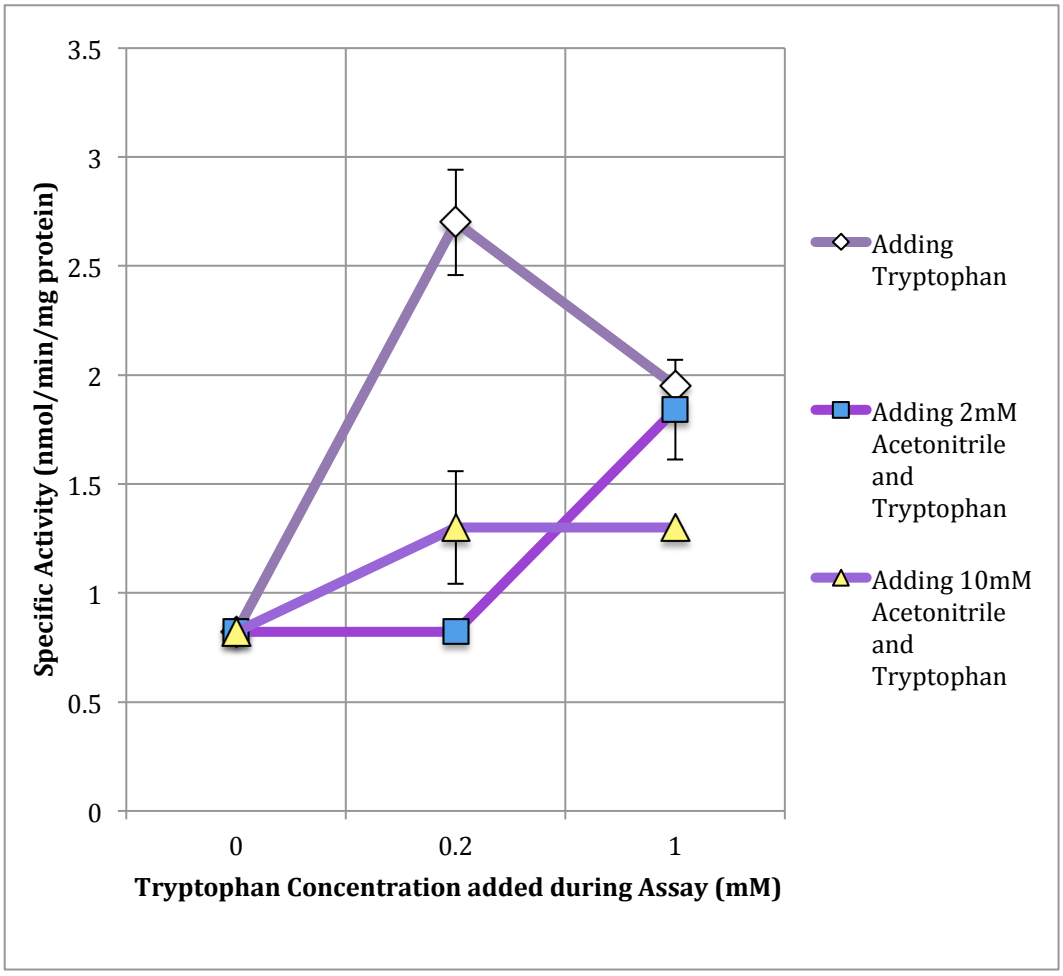
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, 2 mM acetonitrile and 0.2 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 43. Glucose Uracil with 2 mM Acetonitrile and 0.2 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	0.821 ± 0.000	0.821 ± 0.000	0.821 ± 0.000
0.2	2.70 ± 0.242	0.821 ± 0.000	1.30 ± 0.258
1.0	1.95 ± 0.000	1.84 ± 0.228	1.30 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 39. *P. aeruginosa* grown in Glucose Uracil with 2 mM Acetonitrile and 0.2 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, 2 mM acetonitrile and 0.2 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

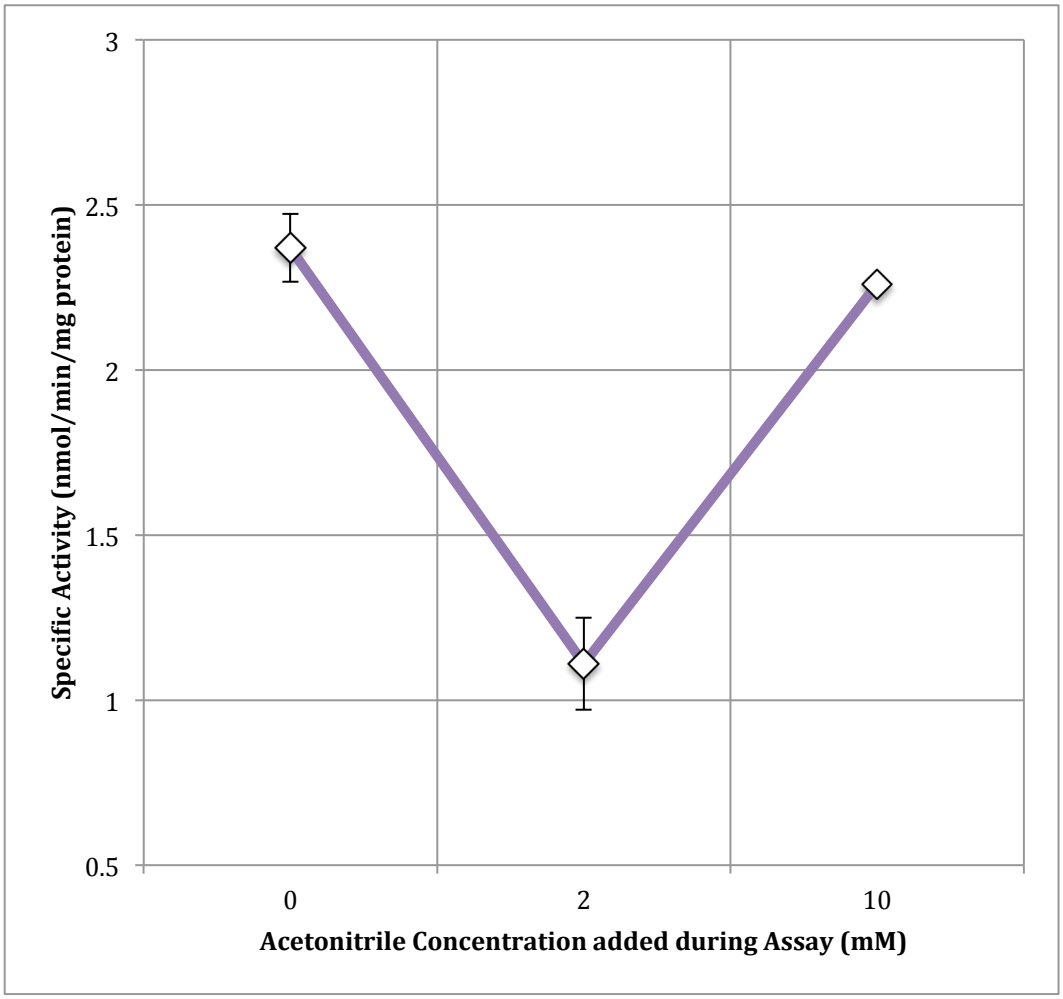
The effects of adding acetonitrile to glucose uracil with 2 mM acetonitrile and 1 mM tryptophan minimal media can be seen in Table 44 and Figure 40. It can be seen in Table 44 that a 2-fold increase in acetonitrile concentration resulted in a 2.1-fold decrease in specific activity and a 10-fold increase in acetonitrile concentration resulted in a 1-fold decrease in specific activity. Adding tryptophan to glucose uracil with 2 mM acetonitrile and 1 mM tryptophan minimal media effects can be seen in Table 45 and Figure 41. It can be seen in Table 45 that a 1-fold increase in tryptophan resulted in a 1.2-fold increase in specific activity when adding tryptophan concentrations, a 3.8-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.8-fold decrease in specific activity when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 45 that a 5-fold increase in tryptophan concentration resulted in a 1.3-fold decrease in specific activity when adding tryptophan concentrations, a 2.1-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 1.7-fold decrease in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 44. Glucose Uracil with 2 mM Acetonitrile and 1 mM Tryptophan Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	2.37 ± 0.103
2	1.11 ± 0.139
10	2.26 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 40. *P. aeruginosa* grown in Glucose Uracil with 2 mM Acetonitrile and 1 mM Tryptophan Minimal Media adding Acetonitrile



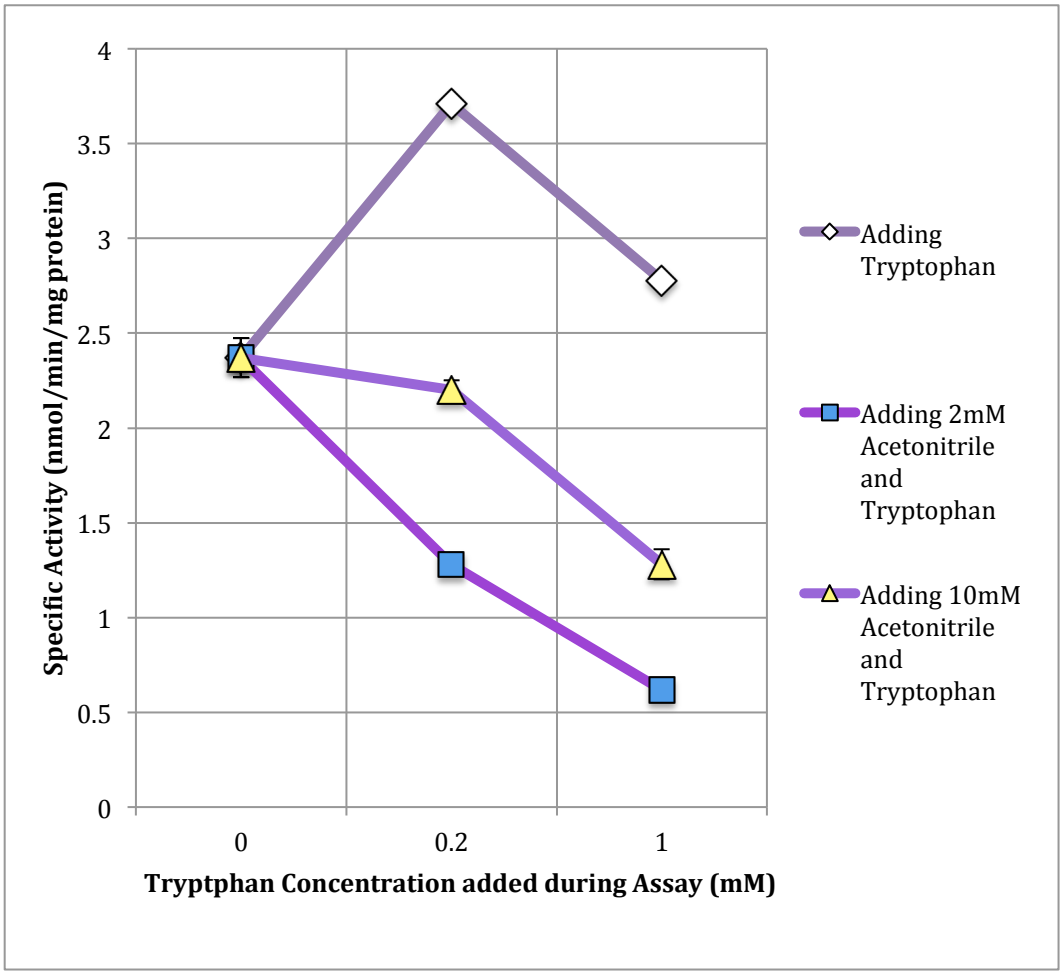
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, 2 mM acetonitrile and 1 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 45. Glucose Uracil with 2 mM Acetonitrile and 1 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	2.37 ± 0.103	2.37 ± 0.103	2.37 ± 0.103
0.2	3.71 ± 0.000	1.28 ± 0.0396	2.20 ± 0.0511
1.0	2.78 ± 0.000	0.618 ± 0.000	1.28 ± 0.0793

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 41. *P. aeruginosa* grown in Glucose Uracil with 2 mM Acetonitrile and 1 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, 2 mM acetonitrile and 1 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

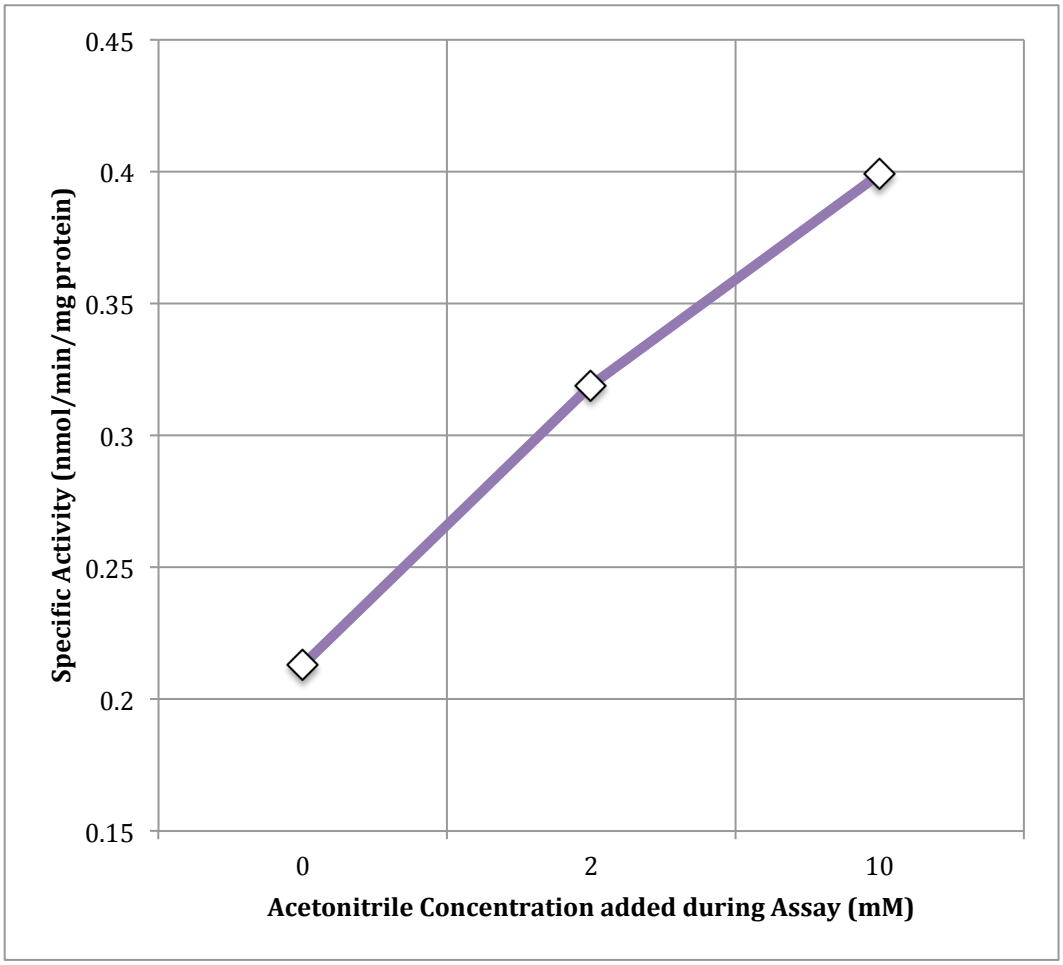
The effects of adding acetonitrile to glucose uracil with 10 mM acetonitrile and 0.2 mM tryptophan minimal media can be seen in Table 46 and Figure 42. It can be seen in Table 46 that a 2-fold increase in acetonitrile concentration resulted in a 2.1-fold decrease in specific activity and a 10-fold increase in acetonitrile concentration resulted in a 1-fold decrease in specific activity. Table 47 and Figure 43 show the effects of adding tryptophan to glucose uracil with 10 mM acetonitrile and 0.2 mM tryptophan minimal media. It can be seen in Table 47 that a 1-fold increase in tryptophan resulted in a 2.2-fold increase in specific activity when adding tryptophan concentrations, a 1.2-fold increase in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 3.6-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 47 that a 5-fold increase in tryptophan concentration resulted in a 1.6-fold decrease in specific activity when adding tryptophan concentrations, a 3.3-fold decrease in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 2.2-fold decrease in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 46. Glucose Uracil with 10 mM Acetonitrile and 0.2 mM Tryptophan Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.213 ± 0.000
2	0.319± 0.000
10	0.399 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 42. *P. aeruginosa* grown in Glucose Uracil with 10 mM Acetonitrile and 0.2 mM Tryptophan Minimal Media adding Acetonitrile



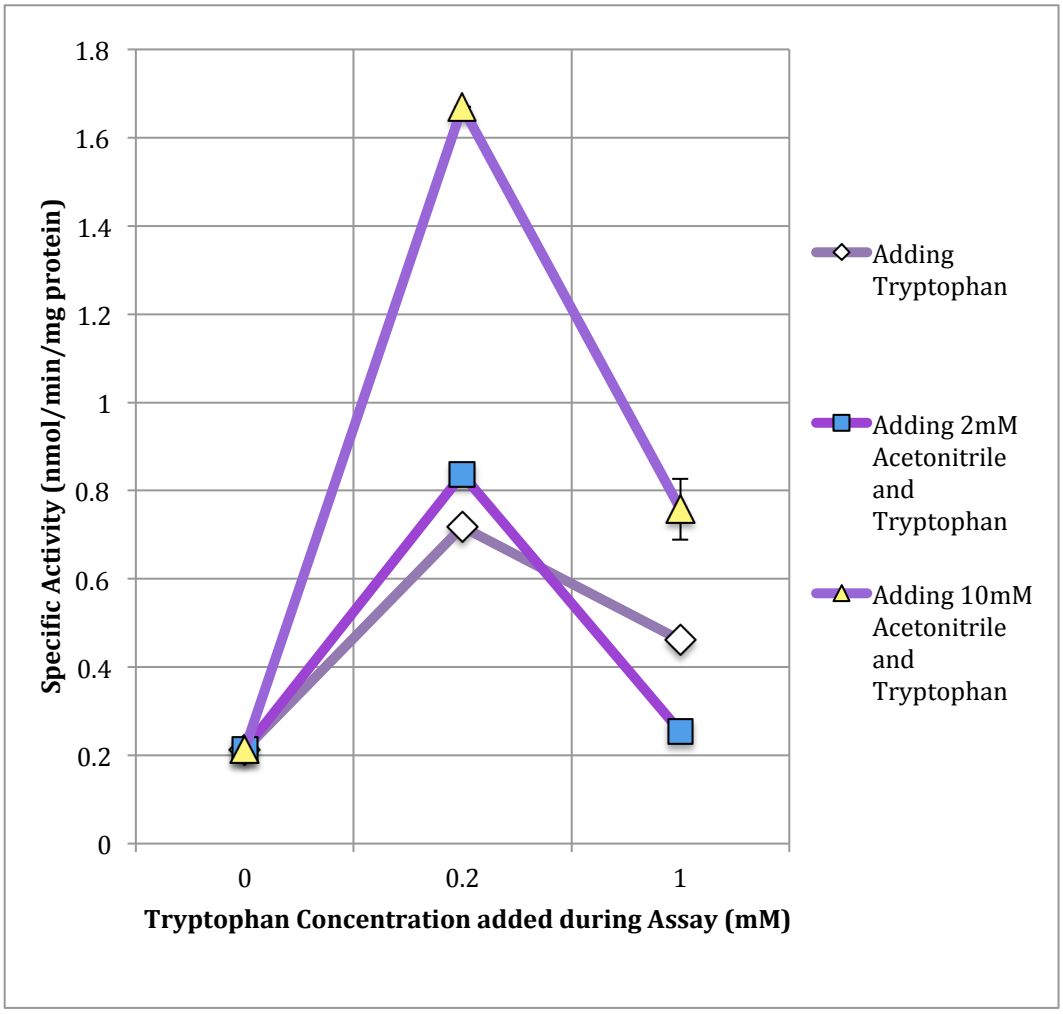
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, 10 mM acetonitrile and 0.2 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 47. Glucose Uracil with 10 mM Acetonitrile and 0.2 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg)
0	0.213 ± 0.000	0.213 ± 0.000	0.213 ± 0.000
0.2	0.718 ± 0.000	0.837 ± 0.0187	1.67 ± 0.000
1.0	0.461 ± 0.0154	0.253 ± 0.0187	0.758 ± 0.0691

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 43. *P. aeruginosa* grown in Glucose Uracil with 10 mM Acetonitrile and 0.2 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, 10 mM acetonitrile and 0.2 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

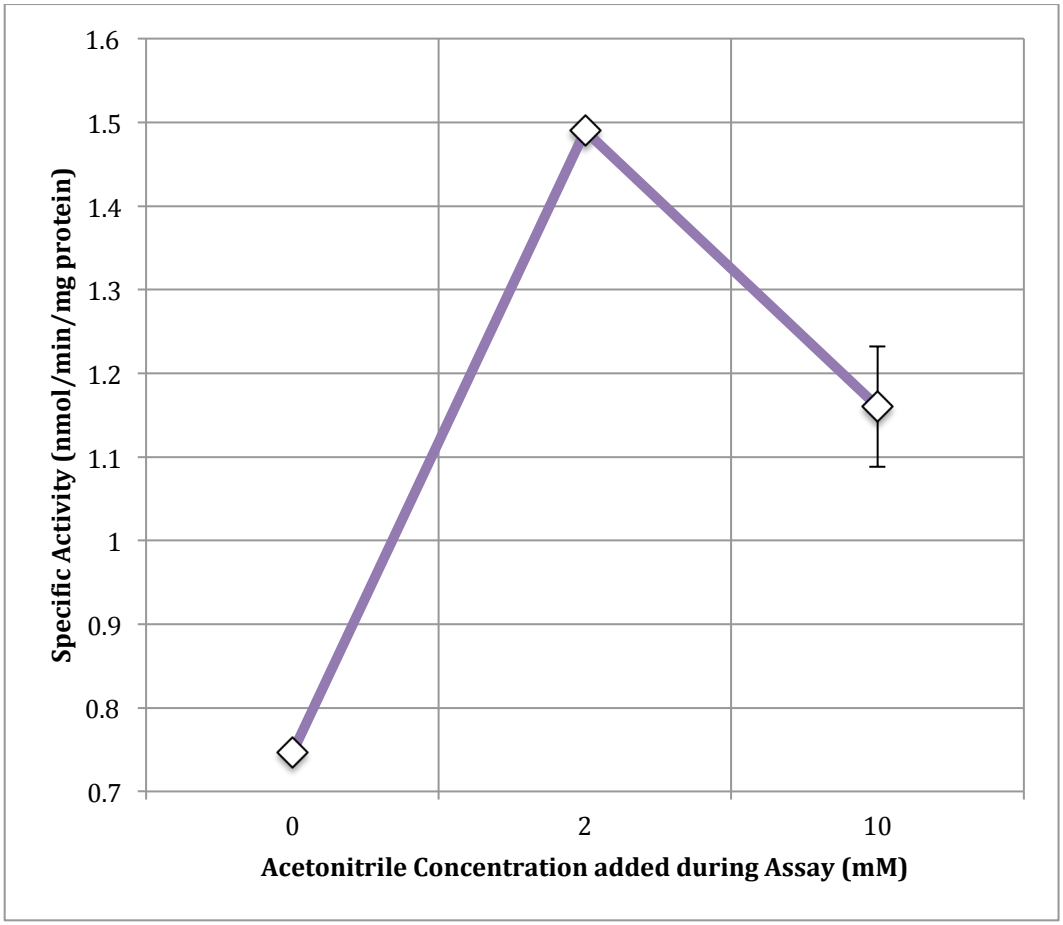
The effects of adding acetonitrile to glucose uracil with 10 mM acetonitrile and 1 mM tryptophan minimal media can be seen in Table 48 and Figure 44. It can be seen in Table 48 that a 2-fold increase in acetonitrile concentration resulted in a 2-fold increase in specific activity and a 10-fold increase in acetonitrile concentration resulted in a 1.6-fold increase in specific activity. Adding tryptophan to glucose uracil with 10 mM acetonitrile and 0.2 mM tryptophan minimal media effects can be seen in Table 49 and Figure 45. It can be seen in Table 49 that a 1-fold increase in tryptophan resulted in no change in specific activity when adding tryptophan concentrations, no change in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and a 2-fold increase in specific activity when adding 10 mM acetonitrile and tryptophan concentrations. Also, it can be seen in Table 49 that a 5-fold increase in tryptophan concentration resulted in a 3-fold increase in specific activity when adding tryptophan concentrations, no change in specific activity when adding 2 mM acetonitrile and tryptophan concentrations, and no change in specific activity when adding 10 mM acetonitrile and tryptophan concentrations.

Table 48. Glucose Uracil with 10 mM Acetonitrile and 1 mM Tryptophan Minimal Media adding Acetonitrile

Acetonitrile Concentration (mM)	Adding Acetonitrile Concentrations Specific Activity (nmol/min/mg protein)
0	0.747 ± 0.000
2	1.49 ± 0.000
10	1.16 ± 0.0719

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 44. *P. aeruginosa* grown in Glucose Uracil with 10mM Acetonitrile and 1mM Tryptophan Minimal Media adding Acetonitrile



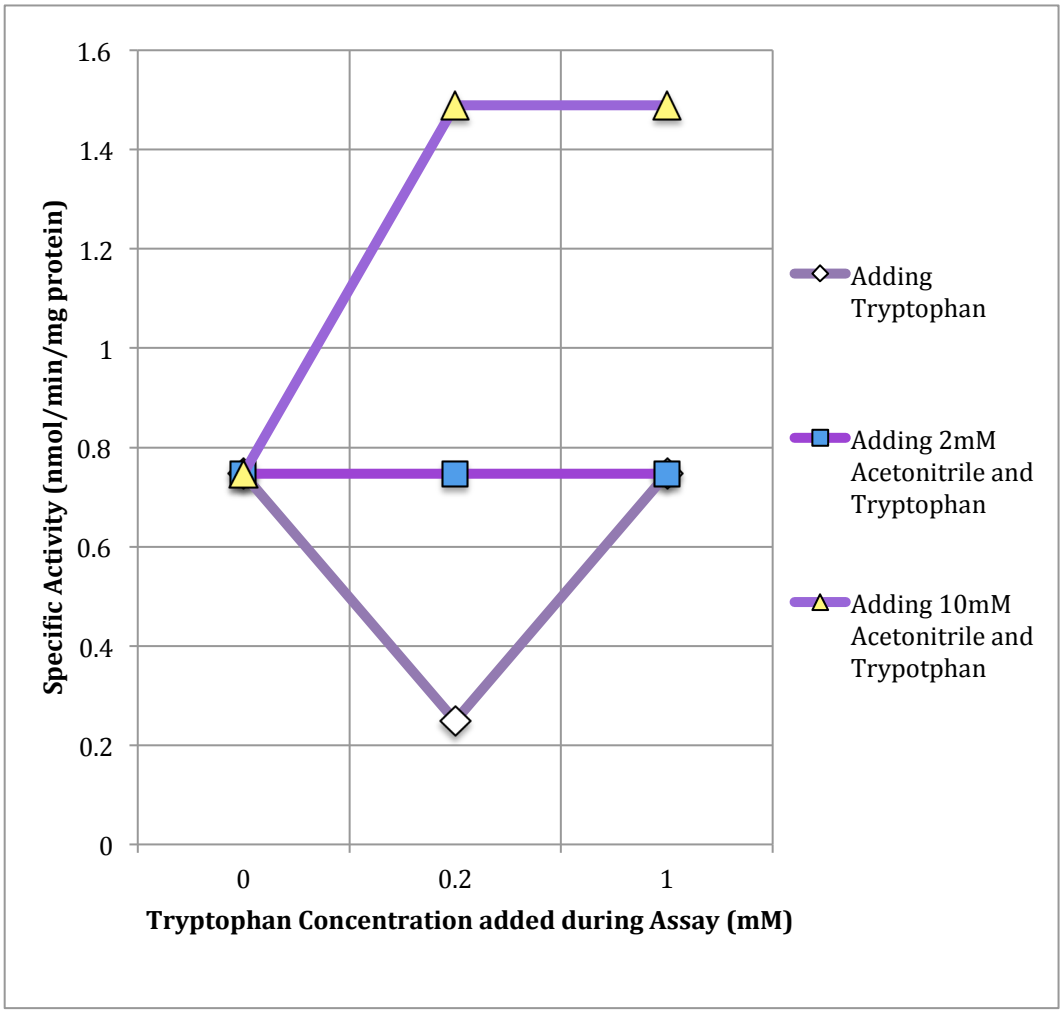
These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, 10 mM acetonitrile and 1 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the acetonitrile concentration. The values were an average of the values [standard deviation].

Table 49. Glucose Uracil with 10 mM Acetonitrile and 1 mM Tryptophan Minimal Media adding Tryptophan

Tryptophan Concentration (mM)	Adding Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 2mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)	Adding 10mM Acetonitrile and Tryptophan Concentrations Specific Activity (nmol/min/mg protein)
0	0.747 ± 0.000	0.747 ± 0.000	0.747 ± 0.000
0.2	0.249 ± 0.000	0.747 ± 0.000	1.49 ± 0.000
1.0	0.747 ± 0.000	0.747 ± 0.000	1.49 ± 0.000

Each specific activity represents the mean of three separate observations (standard deviation).

Figure 45. *P. aeruginosa* grown in Glucose Uracil with 10 mM Acetonitrile and 1 mM Tryptophan Minimal Media adding Tryptophan



These cells were grown in liquid medium containing 0.2% uracil as the source of nitrogen, 0.4% glucose as the carbon source, 10 mM acetonitrile and 1 mM tryptophan. Specific activity was expressed as nanomoles/minute/milligram of protein at 30^{o}C and this is compared to the tryptophan concentration. The values were an average of the values [standard deviation].

Discussion

The purpose of this research was to mimic some conditions that may contribute a color change when simulating the urinary tract catheter in the laboratory. This was done in order to identify if acetonitrile, tryptophan, and/or ammonia have the potential to regulate the rate-limiting step of the pyrimidine metabolic pathway in *P. aeruginosa* ATCC 15692. Assays performed on glucose or succinate as the carbon source and uracil or thymine as the nitrogen source alone served as the control. These values were then used to measure any variation in specific activity of the enzyme, indicating regulation by the trace metal introduced. For the purpose of this research, an increase in specific activity as the concentration is increased is a negative regulator and a decrease in specific activity as the concentration in increased is a positive regulator. For this project, a decrease in specific activity was desired in order to determine if the enzyme could be controlled by the addition of acetonitrile, tryptophan and/or ammonia.

Acetonitrile has been found to act as a negative regulator. It was found that in an acetonitrile solution, the specific activity of chemokines was significantly enhanced (Broxmeyer et al., 1995). Also, it was found that zinc's specific activity in the rat kidney was significantly greater when the rat was fed with a tryptophan diet (Evans & Johnson, 1980). Meaning, that tryptophan is known to act as a negative regulator. If ammonia is inhibiting specific activity, then as higher and higher concentrations of ammonia are added the specific activity will decrease. Indicating that ammonia acted as a positive regulator (Zhou & Qiu, 2006).

When growing *P. aeruginosa* ATCC 15692 in glucose uracil minimal medium, it was found that adding higher and higher concentrations of acetonitrile resulted in the lowering of the specific activity. Whereas, adding increased concentrations of tryptophan and ammonia resulted in the increasing of the specific activity. This demonstrates that acetonitrile can be used as a positive regulator while tryptophan and ammonia can be used as negative regulators.

When growing *P. aeruginosa* ATCC 15692 in glucose or succinate as the carbon source, uracil or thymine as the nitrogen source, and 10 mM acetonitrile, it was found that adding acetonitrile resulted in a variance of change in dehydrogenase specific activity. When adding acetonitrile to the minimal medium with glucose as the carbon source and uracil or thymine as the nitrogen source, the specific activity increased as the concentration of acetonitrile increased. However, when adding acetonitrile to the minimal medium with succinate acting as the carbon source and uracil or thymine as the nitrogen source, the specific activity increased as the concentration of acetonitrile decreased. This demonstrates that acetonitrile will act as a positive regulator when added to glucose based minimal media and act as a negative regulator when added to succinate based minimal media.

When growing *P. aeruginosa* ATCC 15692 in glucose or succinate as the carbon source and uracil or thymine as the nitrogen source, it was found that adding tryptophan along with adding 2 mM acetonitrile resulted in a variance of change in specific activity. When adding tryptophan to the minimal medium with glucose as the carbon source and uracil or thymine as the nitrogen source, the specific activity

increased as the concentration of tryptophan increased. However, when adding tryptophan to the minimal media with succinate as the carbon source and uracil or thymine as the nitrogen source, the specific activity increased as the concentration of tryptophan decreased. This is consistent with the findings found when adding acetonitrile to these minimal media. This demonstrates that tryptophan will act as a negative regulator when added to glucose based minimal media and act as a positive regulator when added to succinate based minimal media.

When growing *P. aeruginosa* ATCC 15692 in succinate as the carbon source, uracil or thymine as the nitrogen source, and 10 mM acetonitrile, it was found that adding 2 mM acetonitrile and tryptophan concentrations resulted in the reverse affect of adding 10 mM acetonitrile and tryptophan concentrations to the minimal media. When adding tryptophan and 2 mM acetonitrile to succinate uracil or succinate thymine with 10mM acetonitrile minimal media, it resulted in an increase in specific activity when the concentration of tryptophan decreased. However, when adding tryptophan and 10 mM acetonitrile to succinate uracil or succinate thymine with 10 mM acetonitrile minimal media, it resulted in an increase in specific activity when the concentration of tryptophan increased. Therefore, tryptophan, being added to succinate uracil or succinate thymine with 10 mM acetonitrile minimal media, acts as a positive regulator when added in addition with 2 mM acetonitrile and acts as a negative regulator when added in addition with 10 mM acetonitrile.

When growing *P. aeruginosa* ATCC 15692 in glucose uracil, succinate uracil, or glucose thymine with 1 mM tryptophan minimal media and adding 2 mM

acetonitrile and tryptophan concentrations, it was found that this resulted in the same effect. The specific activity increased from adding 0.2 mM tryptophan to adding no tryptophan to the enzyme assay and the specific activity increased from adding 0.2 mM tryptophan to adding 1.0 mM tryptophan to the enzyme assay. This demonstrates that tryptophan can act as a regulator when also adding 2 mM acetonitrile due to the fact that they all resulted in the same increasing or decreasing specific activity.

When growing *P. aeruginosa* ATCC 15692 in glucose uracil with 2 mM acetonitrile and 0.2 mM tryptophan and glucose uracil with 10 mM acetonitrile and 1 mM tryptophan minimal media, it was found that when adding 2 mM acetonitrile and tryptophan concentrations and when adding 10 mM acetonitrile and tryptophan concentrations there were five observations where there was no change in specific activity. Since there were only eight observations, it was shown that that over half of the time there was no change in specific activity. This demonstrates that we cannot detect tryptophan, when also adding 2 mM acetonitrile and 10 mM acetonitrile in those minimal media, acting as a regulator.

Throughout this project, it was found that adding 2 mM acetonitrile and adding 1 mM tryptophan during the enzyme assay to minimal media containing glucose as the carbon source and uracil as the nitrogen source, the specific activity increased when compared to those minimal media when not adding compounds during the enzyme assay. This was the case except for when adding 2 mM acetonitrile and 1 mM tryptophan during the enzyme assay to minimal media containing glucose as the carbon source, uracil as the nitrogen source, 2 mM or 10 mM acetonitrile, and

1 mM tryptophan. This might be the case, because uracil may not be degraded and these minimal media might act as an inhibitor. Whereas, all other glucose uracil based minimal media when adding 2 mM acetonitrile and 1 mM tryptophan during the enzyme assay may be rapidly degrading uracil, which increases the specific activity. In these media, this might be occurring due to its need to breakdown uracil to use in another pathway. All the data obtained in this research shows that the enzyme pathway can indeed be regulated by the addition of acetonitrile, tryptophan and ammonia not only by adding these compounds during the enzyme assay, but also by growing the bacterium in them.

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