Market Analysis of a Naphthenic Acid Biosensor

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Introduction

The oil sands of northern Alberta are one of the largest crude oil reserves in the world and account for approximately 95% of Canada's oil reserves.[1] However, during the hot-water extraction process of bitumen from the oil sands, containing heavy metals, sand, fine clay particles, hydrocarbons, and naphthenic acids. Under the "zero-discharge" policy implemented by the Alberta government, this wastewater is sequestered into large artificial settling ponds known as tailings ponds. Naphthenic acids (NAs) are a class of carboxylic acid compounds found in these tailings ponds that are known to be toxic to wildlife and aquatic life at low concentrations.[2]

Due to their toxicity, there is a need in the market for measuring levels of NAs to ensure the safety of the environment and individuals who work with the tailings ponds. Fortunately, there are a number of microbial species indigenous to the tailings ponds that are capable of biodegrading NAs.[2] The University of Calgary iGEM team is developing a naphthenic acid biosensor using NA-sensing elements from these species. This inexpensive, portable biosensor is capable of producing a rapid and quantifiable electrochemical output, which can be used as a method of measurement even in the murkiest of tailings samples.

Operation of the Biosensor

Using the naphthenic acid biosensor will be simple and efficient. The device consists of a single chamber which holds a replaceable, disposable cartridge containing dried bacteria and the active chemical used for detection. When a sample of tailings water is added into the chamber, the bacteria will rehydrate and the chemical will dissolve. The user will then insert a clean, disposable electrode into the water sample. This electrode is connected to a potentiostat, which will supply a voltage where, within a few minutes, a computer can record the output.

This is a procedure that can be followed by any individual with basic training, and instructions will be provided with the kit. Measured samples can be treated with bleach, 70% ethanol, or autoclaved before disposal.

The naphthenic acid biosensor will not require extensive technical qualifications for its operation, nor will it require pre- and post-processing of the tailings water samples. This will allow for a rapid and portable device that can measure levels of NAs on demand.

Competitive Analysis

The current industry standard for the analysis of NAs in tailings samples is performed using Fourier Transform infrared spectrometry (FTIR).[3] FTIR involves a filtered acidified sample of tailings that is put into a rotarytumbler apparatus, using dichloromethane to extract NAs. The absorbance of the NAs sample is then compared to commercially available NA mixtures of known concentration. This particular quantification method measures the amount of carboxyl groups in the sample. Since the composition of the commercial mixture is not likely to match that of the sample, there remains some bias in the total NA value of the sample.[4] In a conversation with a representative from Maxxam Analytics (Oct 2011), we learned that this method is capable of detecting resolutions to one milligram per litre total naphthenic acids, though other sources indicate that FTIR can be sensitive enough to detect a few tenths of a milligram per litre.[3]

Another method of testing for NAs involves the application of negative-ion electrospray ionization-mass spectrometry (ESI-MS).[3] This involves extraction of aqueous NAs using a divinyl benzene sorbent and acetonitrile. The samples are then ionized and sent through a mass spectrometer, which records peaks depending on how the acids fragment. This method is capable of detecting samples containing NAs as low as 0.01 mg/L.[5]

Analytical service companies charge anywhere between $200 to $500 dollars per sample for a NA water analysis.[6] This price may vary based on the amount of business supplied by the client, and so would be less for those companies practicing regular testing/analysis. The standard turnaround time is approximately 5 business days. Further details will be shown in the Cost Analysis section below.

In comparison to these methods, our biosensor will be capable of reporting levels of NAs within minutes. This can shorten the testing time from almost a week to potentially only a few hours maximum, depending on the number of samples required to be tested. As well, it will not require treating the tailings pond water samples to extract NAs prior to testing. Extraction processes are not always efficient and therefore some NAs may be inaccurately measured. For example, the preparation of ESI-MS testing is highly dependent on pH which can affect extraction efficiency.[5] Instead, our device will measure NA levels in tailings ponds water directly, without the need to pre-treat the sample.

There are other existing biosensors on the market, such as those that detect heavy metals[7] and polycyclic aromatic hydrocarbons.[8] These devices function similarly to our biosensors, but are designed for the detection of different compounds and use luminescent output, which is not optimal for turbid tailings environments. Our device produces an electrochemical output that can be measured regardless of what the tailings water sample looks like. There are no biosensors for NAs currently available in the market, which shows an available niche for our device. However, other sensors, such as one that uses UV spectrometry, are currently under development.[9]

Cost Analysis

In order to determine the cost-effectiveness of our naphthenic acid biosensor, we must compare it to existing methods in the market, namely FTIR and ESI-MS. In Alberta, it is mandated by the Environmental Protection and Enhancement Act that NAs in tailings ponds be measured at least once per year.[10] However, there is pressure from various groups to add NAs to the National Pollutant Release Inventory, which would more tightly regulate the level of NAs permitted to be released into tailings ponds annually.[12] Thus, there is good reason for oil sands operators to monitor levels throughout the year.

Tailings ponds are rather large and NA concentrations are presumably heterogeneous throughout the ponds. Thus, multiple samples are required in order to get a thorough and accurate measurement of NA levels. There are currently approximately 170 square kilometres of tailings ponds in the northern Alberta oil sands region.[13] However, the surface area of individual tailings ponds are more difficult to determine because of the varying sizes of mining projects. Since NA concentrations can also vary with depth, a three-dimensional assay of tailings ponds can give the best overall average NA measurement.

Due to the high variability of NA concentrations within tailings ponds, a higher number of samples spaced closer together will give a more accurate view of overall NA concentrations in the pond. We assume that oil sands operators will need to take at least 3 samples at every sampling point, and 4 sample points for every square kilometre of tailings pond. This would mean that 2040 total samples will need to be taken to measure the 170 square kilometres of tailings ponds. Even at the low end of the price scale at $200 per assay, the total cost of sampling will be no less than $408,000.

We assume that there are approximately 7-10 major corporations running large-scale projects in the Alberta oil sand region.[14] Therefore, we shall estimate that the final cost of analysis will be split evenly amongst 10 major corporations, though individual companies may be spending significantly more or less on testing. Even so, the minimum each company must pay would be approximately $40,800 for extremely sporadic sampling in their own tailings ponds.

A higher resolution test could involve take 5 to 10 samples at varying depths at each sampling point, with one sampling point every hectare (or 100 sampling points per square kilometre). This would mean there would be 500 to 1000 samples that are taken for every square kilometre. At the lowest available cost of $200 per assay, this would result in upwards of $34 million dollars expenditure for the testing of NAs in tailings ponds. Depending on the size of the project, this can be even more costly.

We can compare this cost to the proposed production and usage cost of the NA biosensor. A major advantage of electrochemical detection versus luminescent or other light-based detection is its relatively simple instrumentation. The biosensor can be constructed using typical industrial components. Here is a breakdown of the cost of production for the base unit without the replaceable components:

Component	**Price Per Unit ($)**	**Units**	**Cost (CAN $)**
Prototyping Board	13.70	1	13.70
Box	9.90	1	9.90
Batteries and Connectors	2.64	2	5.28
Electrode Connectors	3.23US	1	3.24
Other Components	1.09US	3	3.27
Wires	18.06US/30.5m	25cm	0.15
		Total Cost	35.54

The replaceable components include creating a cartridge containing both an electrode and an amount of freeze-dried bacteria. The electrodes can be purchased in packages of 100 for $200 from companies such as Pine Research Instrumentation.[15] This means that each individual electrode is only $2. The bacteria can be grown in a type of medium known as Lysogeny Broth (LB). Biotechnology companies such as Invitrogen Life Sciences sell LB in containers of 2.5kg for approximately $300.[16] Only 20g of medium powder is required for a litre of medium. Therefore a single container of LB can yield up to 125 litres of medium. The biosensor does not require many bacteria in order to produce a quantifiable output. So long as the concentration of cultured cells is sufficient, the volume of bacteria to be freeze-dried can be as low as 1 mL, if not less. Assuming we freeze-dry 1 mL of cells for every cartridge, one container of LB broth can produce up to 125,000 cartridges worth of freeze-dried bacteria. If we divide the original cost of the LB by this number, the original cost of the media is $0.002 per cartridge, which is a negligible expense.

In order to manage, culture, and grow these cells, lab technicians will need to be employed. We will assume that a stipend of $25 per hour will be paid for a lab technician, based on typical wages for such a position.[17] We estimate that a lab technician should, for each hour, be capable of producing, testing, and aliquoting approximately 200 mL of cells. If the distribution of cells is mechanized or automated, this production rate can be even faster. This would mean that a minimum of 200 cartridges can be produced with one hour of work.

The cost of the replaceable components can be summarized here:

Component	Total Cost ($)	Units	Cost Per Unit ($)
LB Broth for Cultures	333.00	125 000	0.0027
Electrodes	200.00	100	2.00
Lab Technician Stipend	25.00/hr	200	0.13
		Total Cost	2.13

Factoring in variable overhead costs such as a casing for the cartridges, we can sell these cartridges for prices as low as $5. This means that with 1000 samples per square kilometre, even if a single company tested all 170 square kilometres of tailings in the sampling scheme above, it would only cost $850,000, plus the cost of the base instrument. Since the cost to construct the base instrument is approximately $40, this is a negligible price in the long run. For each company, the biosensor method is 40 times less expensive than testing at the lowest price point via traditional methods. Even if we marked up the price of the cartridges by 400% to a price of $20, this would still be one-tenth the cost of competing methods. If used as a preliminary screening, 1000 samples per square kilometre can be inexpensively screened using the NA biosensor and any particular samples of interest can be further examined in higher detail using FTIR or ESI-MS.

This comparison does not take into account the difference in paying lab technicians for training, certification, and salary, nor does it take into consideration time or shipping costs. Our NA biosensor does not require highly trained and qualified specialists to run tests on tailings water. Any individual who understands basic work safety and can follow the provided instructions is capable of performing these tests. Results will be generated in minutes, rather than the standard 5-business-day turnaround.[6]

Analysis of Potential Markets

We believe that the oil and gas industry in Alberta would be the ideal market to target first, since the oil sands in northern Alberta supplies approximately 95% of all Canada's crude oil.[1] The largest oil and gas companies in Canada and the United States possess over $300 billion in assets, though smaller companies possess approximately $10 to $20 billion in assets.[11] If we consider the estimated costs of NA measurement as seen in the Cost Analysis above, it does not appear that such costs greatly impact large- or medium-sized companies financially. However, the millions of dollars put into testing annually can instead be put into testing multiple times a year in order to ensure compliance with government standards. This will also provide oil companies with a more positive image from the public, as increased testing shows an improved commitment to maintaining and preserving the environment.

Alternatively, instead of targeting larger oil corporations, it would potentially be more beneficial for smaller entrepreneurial oil companies or contracted analysis companies to conduct these tests using an inexpensive and rapid biosensor. There is a "zero-discharge" policy set in place for tailings ponds, which requires that tailings be stored on-site.[10] There are also mandates set in place by the Alberta government that water seepage from tailings ponds must not occur.[13] Another application of the NA biosensor, therefore, is to test areas surrounding tailings ponds, such as nearby water sources, for NAs. Scientific programs such as the Regional Aquatics Monitoring Program (RAMP) measure levels of toxins in the many bodies of water such as rivers, creeks, and lakes upstream and downstream of oil sands areas. These include, but are not limited to, the Lower Athabasca River, major watersheds and basins leading to the River, wetlands, and lakes.[18] Measurements must be taken once every season.[19] These samples are all analyzed in external laboratory facilities, and thus have all of the associated costs attached to sending samples off-site.[18] However, it has been stated in the RAMP 2010 Technical Report that a more standardized method of NA testing for routine use must be established since multiple analytical facilities yield different results. This is due primarily to these facilities detecting non-NA hydrocarbons or only detecting levels of specific NAs, which does not provide an accurate overall picture of NA levels.[18]

This is the primary market that the biosensor may be able to enter: the need for a more standardized testing method which yields more standardized results. Our NA biosensor will detect overall levels of NAs regardless of what the structure of each individual component compound is. Therefore, the biosensor will be capable of providing a more standardized measurement of NAs in samples of tailings water or freshwater. Standardizing NA measurement between different tailings ponds, rather than comparing the varying standards of different analytical companies, will be beneficial to the long-term analysis and monitoring of such environmental toxins. Therefore, by introducing the biosensor as a government measurement standard, this can greatly increase revenue.

Marketing Strategy

The oil and gas industry is very well-established, which means that introducing a naphthenic acid biosensor will involve some degree of perceived risk. We must therefore tackle the market in phases, with extensive research in each phase. We must plan how this product will be distributed to companies and eventually how this may be established as a standardized method of detecting NAs.

The first target will most likely be the small entrepreneurial oil and gas companies in Alberta. We believe that these companies are the best trial market for our NA biosensor primarily because there are thousands of small businesses stationed in Alberta that provide many services to the oil sands projects.[20] We intend to target specifically companies that specialize in the measurement and analysis of tailings water samples for larger oil corporations. These smaller companies are likely more accepting of rapid, inexpensive technologies as they may not possess the same amount of wealth as large corporations. In this phase, we intend to directly approach such small companies and present the benefits of using our NA biosensor. We will distribute trial kits for these companies to use and record any feedback they may have on how we can improve the product. By doing so, we hope to make any revisions before expanding to the next phase. This phase will likely last 2-5 years, depending on the feedback that we receive.

This cycle of selling, distribution, and feedback will allow us to continue to distribute to other companies in Alberta. We hope to grow and eventually target larger oil and gas corporations. At this point, we will have a number of smaller oil companies who are satisfied with the performance of our biosensor and thus may help us encourage the larger companies to also adapt to this method of measurement. We hope to begin contacting and reaching representatives from larger corporations within the next 5 years.

Simultaneously, we wish to lobby the Canadian federal government and Alberta provincial government to adapt the NA biosensor as a standardized method of measuring levels of NAs in tailings ponds. We will contact representatives from Environment Canada and Alberta Energy to obtain information on how we may go about establishing such a standard. This will be an ongoing process and is a long-term goal for our product.

Conclusion

Currently there is increasing pressure on oil sands companies to monitor and manage potential environmental toxins from their projects. Tailings ponds harbour large quantities of wastewater that contain toxins which can potentially leach into nearby water sources. There is currently a push for government agencies to establish naphthenic acids as potentially toxic compounds that must be monitored on a regular basis in both the tailings ponds and the surrounding environment. However, for many smaller companies, shipping the large number of samples required for analysis to other analytical facilities is expensive and time-consuming. A naphthenic acid biosensor is a cheap and rapid alternative to existing methods of measuring NAs. We intend to market this sensor initially to these smaller companies as an inexpensive preliminary screen. Because there is a need in the market for a more standardized method of measuring levels of NAs, we also intend to eventually establish the biosensor as such a standardized technique. This way, tailings ponds from multiple different sites can be compared on the same standard and provide more accurate analysis of NAs in different samples.

References

1. Government of Alberta. Alberta Energy: Facts and Statistics [Internet]. Cited 2011 Oct 7. Available from: http://www.energy.alberta.ca/OilSands/791.asp

2. Whitby C. Microbial Naphthenic Acid Degradation. in: Laskin AI, Sariaslani S, Gadd GM, editors. Advances in Applied Microbiology. Vol 70. Burlington. Academic Press; 2010; pp. 93-125.

3. Clemente JS, Fedorak PM. A Review of the Occurrence, Analyses, Toxicity, and Biodegradation of Naphthenic Acids. *Chemosphere* 2005; 60:585-600.

4. Jivraj MN, MacKinnon MD, Fung B. Naphthenic Acid Extraction and Quantitative Analysis with FTIR Spectroscopy. Technical Report. Edmonton (AB): Syncrude Research Centre; 1995 Oct.

5. Headley JV, Peru KM, McMartin DW, Winkler M. Determination of Dissolved Naphthenic Acids in Natural Waters by Using Negative-Ion Electrospray Mass Spectrometry. *Journal of AOAC International* 2002; 85(1):182-7.

6. CanmetENERGY. List of Analytical Services and Prices [Internet]. 2011 [updated 2009 Jun 04; cited 2011 Oct 21]. Available from: http://canmetenergy-canmetenergie.nrcan-rncan.gc.ca/eng/about_us/devon/analytical_pilot_facilities/analytical_services.html

7. Verma N, Singh M. Biosensors for Heavy Metals. *BioMetals* 2005; 18:121-9.

8. Crown Biotechnology Ltd. The Safe Soil Tester™ [Internet]. Cited 2011 Oct 22. Available from: http://crownbio.co.uk/products/

9. Taschuk MT, Wang Q, Drake S, Ewanchuk A, Gupta M, Alostaz M, Ulrich A, Sego D, Tsui YY. Portable Naphthenic Acid Sensor for Oil Sands Applications. Paper presented at: 2nd International Oil Sands Tailings Conference 2010; 2010 December 5-8; Edmonton, Alberta.

10. Government of Alberta. Environmental Protection and Enhancement Act R.S.A. 2000, c.E-12, as amended. Calgary, AB; 2004 Apr 6. 56p. Approval No.: 149968-00-01.

11. Forbes. The World's Biggest Public Companies [Internet]. 2011 [updated 2011 Apr; cited 2011 Oct 22]. Available from: http://www.forbes.com/global2000/

12. Environmental Defence. Proposal by Environmental Defence to Add Naphthenic Acids to the NPRI [Internet]. 2010 Nov 11 [Cited 2011 Oct 22]. Available from: http://www.ec.gc.ca/inrp-npri/default.asp?lang=en&n=AC708134-1

13. Government of Alberta. Facts about Alberta's Oil Sands [Internet]. 2011 [updated 2011 Feb; cited 2011 Oct 22]. Available from: http://www.oilsands.alberta.ca/FactSheets/Tailings_Management.pdf

14. Government of Alberta. Alberta Oil Sands Industry Quarterly Update Spring 2011. Alberta; 2011 Mar. 16p.

15. Pine Research Instrumentation. Screen Printed Electrodes (Carbon) [Internet]. Cited 2011 Oct 25. Available from: http://www.pineinst.com/echem/viewproduct.asp?ID=46564

16. Invitrogen. LB Broth Base, powder (Lennox L Broth Base)® [Internet]. Cited 2011 Oct 25. Available from: http://products.invitrogen.com/ivgn/product/12780029?ICID=search-product

17. Government of Alberta, Employment and Immigration. Alberta Occupational Profiles: Biological Technician [Internet]. 2008 [updated 2010 Jan; cited 2011 Oct 25]. Available from: http://alis.alberta.ca/OCCINFO/content/RequestAction.asp?aspAction=GetHTMLProfile&format=html&occPro_ID=71001558&SNT_ID=25

18. Regional Aquatics Monitoring Program. 2010 Technical Report Final. Technical report. Alberta: Regional Aquatics Monitoring Program; 2010 Apr. Report No.: RAMP1565.

19. Regional Aquatics Monitoring Program. Water Quality [Internet]. Cited 2011 Oct 25. Available from: http://www.ramp-alberta.org/ramp/design+and+monitoring/components/water+quality.aspx

20. Alberta's small businesses thrive in shadow of big oil. The Vancouver Sun [Internet]. 2011 Oct 20 [cited 2011 Oct 27]; Available from: http://www.vancouversun.com/business/Alberta+small+businesses+thrive+shadow/5572272/story.html