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CONTACTO

Jéssica Duchicela PhD.
Universidad de las Fuerzas Armadas ESPE
vinculos@espe.edu.ec
http://journal.espe.edu.ec/index.php/vinculos
Tel: (593) 2 3989400 Ext. 2521
Av. General Rumiñahui S/N y Paseo Escénico Santa Clara.
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Carta al editor:
La Academia y los objetivos de desarrollo sostenible

MARCO V. MONCAYO MIÑO
Organización de las Naciones para la Alimentación y la Agricultura
marco.moncayo.mino@gmail.com

"La tierra no la hemos heredado de nuestros ancestros, la tenemos prestada de nuestros hijos" este antiguo proverbio nos permite reflexionar sobre los efectos que las generaciones pasadas han ocasionado en nuestra situación actual, y nos advierte sobre el impacto de nuestras acciones en la realidad de las futuras generaciones. Tomando conciencia de los retos globales a nivel ambiental, económico y social, los líderes de 193 países aprobaron en el año 2015 la Agenda 2030 sobre el Desarrollo Sostenible como una hoja de ruta con objetivos claros y ambiciosos, que van desde erradicar la pobreza, el hambre y proteger el planeta, hasta asegurar la prosperidad e igualdad sin dejar a nadie atrás. La Agenda cuenta con 17 Objetivos de Desarrollo Sostenible (ODS), cada objetivo tiene metas específicas que deben alcanzarse con el esfuerzo de toda la sociedad: los gobiernos, el sector privado, la sociedad civil y la academia. Estos lineamientos globales ofrecen una oportunidad para que las sociedades emprendan un camino que les permita satisfacer sus necesidades y asegurar los recursos para las futuras generaciones.

Después de tres años de haber establecido este desafío global para que todos los gobiernos tomen las riendas de su propio desarrollo, se han obtenido grandes avances. En general las personas viven mejor que hace una década. Sin embargo, estos esfuerzos no han sido suficientes para seguir con el ritmo de cumplimiento de la Agenda 2030. En el 2018 los jóvenes tuvieron tres veces más posibilidades de estar desempleados que los adultos. La desigualdad en los ingresos aún es dominante, los hombres ganaron un 12,5% más que las mujeres. En el aspecto nutricional, tras un prolongado descenso de la prevalencia de subalimentación, por tercer año consecutivo se registra un aumento del hambre a nivel global, este número aumentó de 777 millones en 2015 a 815 millones de personas en 2017. Asimismo en 2017, la temporada de huracanes del Atlántico Norte fue la más costosa de la historia, y la temperatura media mundial de los últimos 5 años fue la más alta registrada[1].

Es evidente que todavía queda mucho trabajo por hacer para alcanzar los ODS. En este aspecto la academia es un actor que puede aportar de forma transversal en los 17 objetivos, es un actor neutral que provee información basada en evidencia y cuenta con credibilidad dentro de la sociedad. Sus principales herramientas son el aprendizaje y la enseñanza, la investigación, la innovación, la cultura y el liderazgo. La universidad debe jugar un rol activo y significativo en la construcción de capital social y el desarrollo de su propio país, y por lo tanto en el alcance de las metas globales.

La revista Vínculos ha dedicado dos números para trabajos de investigación relacionados con 9 de los 17 ODS. Considerando la realidad del Ecuador, se presentan artículos sobre el disminución de la pobreza (objetivo 1), economía (objetivo 8) cooperativismo (objetivo 17), el medio ambiente (objetivo 13) y ecosistema terrestre (objetivo 15), educación (objetivo 4), reducción de la desigualdad (objetivo 10), paz (objetivo 16) y energía renovable (objetivo 7). Estos artículos aportan información valiosa sobre las acciones que se realizan en el país a nivel investigativo y generan evidencia útil para contribuir en los procesos de toma de decisiones que buscan el desarrollo sostenible.

Espero que el importante esfuerzo que realiza la revista Vínculos con este número dedicado a los ODS, sea un detonante para que la academia tome conciencia sobre el relevante rol que juega en el cumplimiento de la Agenda 2030 en el Ecuador.

1 https://unstats.un.org/sdgs/files/report/2018/TheSustainableDevelopmentGoalsReport2018-ES.pdf

OBJETIVOS
DE DESARROLLO
SOSTENIBLE

ARTÍCULO DE INVESTIGACIÓN

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Mejoramiento de condiciones laborales del personal de la Asociación de recicladores Romerillos en Ecuador

Improvement of labor conditions of personnel of the Asociacion de recicladores Romerillos in Ecuador

*TANIA CRISANTO PERRAZO[a], PATRICIO QUEZADA MORALES[b], BORYS CULQUI CULQUI[b], JOSÉ PEREZ ROSALES[b], GABRIEL VACA VILLAMAR[b]

[a] Departamento de Ciencias de la Tierra y la Construcción, Universidad de las Fuerzas Armadas "ESPE", Av. General Rumiñahui S/N y Paseo Escénico Santa Clara, Sangolquí, Ecuador
[b] Departamento de Ciencias de la Energía y Mecánica, Universidad de las Fuerzas Armadas "ESPE", Av. General Rumiñahui S/N y Paseo Escénico Santa Clara, Sangolquí, Ecuador
*ttcrisanto@espe.edu.ec

RESUMEN

El presente trabajo se desarrolló en el contexto de la vinculación con la sociedad, mediante la coordinación entre el Municipio del Cantón Mejía-Ecuador, la Universidad de las Fuerzas Armadas-ESPE y la Asociación de Recicladores de Desarrollo Social Romerillos. Se plantearon como objetivos el mejoramiento de las condiciones laborales del personal de la Asociación, la capacitación del personal, el mantenimiento de equipo existente y el planteamiento de un proceso para agregar valor a las actividades que realizan. Estos objetivos se lograron a través de estrategias del manejo de residuos inorgánicos reciclables acordes a las necesidades, recursos y cultura de la comunidad. Como resultados de este proceso se obtuvo al personal de la Asociación de Recicladores capacitados en Seguridad y Salud Ocupacional, en base a normas de la legislación ecuatoriana, , la implementación de soluciones tecnológicas para el correcto funcionamiento y empleo de la maquinaria existente, garantizando su operatividad y disponibilidad, y se brindó una alternativa para dar valor agregado al proceso de reciclaje de los residuos plásticos específicamente el PET (polietileno tereftalato) y el PEHD (polietileno de alta densidad) mediante la elaboración de ecopostes.

Palabras clave:

Reciclaje, residuos plásticos, ecoposte, condiciones laborales

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ABSTRACT

The present article summarizes the results of an outreach project of Universidad de las Fuerzas Armadas-ESPE developed with Municipality of Canton Mejía-Ecuador, specifically, with the "Asociación de Recicladores de Desarrollo Social Romerillos". The overreaching goal of this project was to improve the working conditions of the Association's workers trough training, maintenance of existing equipment and adding value to the activities they carry out. These objectives were achieved through strategies for the management of recyclable inorganic waste according to the needs, resources, and culture of the community. As a result of this process, the personnel of the Association of Recyclers trained in Occupational Health and Safety was obtained, based on the norms of Ecuadorian legislation, existing machinery in operating conditions, technological solutions for the correct use of the existing machinery, guaranteeing its operability and availability, and an alternative was provided to give added value to the recycling process of plastic waste, specifically PET (polyethylene terephthalate) and PEHD (high-density polyethylene) through the development of ecopostes.

Keywords: Recycling, plastic waste, ecostake, working conditions.

INTRODUCCIÓN

La mayoría de las ciudades latinoamericanas no recolecta la totalidad de los desechos sólidos generados, y sólo una fracción recibe una disposición final adecuada. La sociedad humana siempre ha generado desechos resultantes de los procesos de producción y consumo para satisfacer sus necesidades. Tarde o temprano, los recursos naturales extraídos de bosques, minas, pozos, mantos acuíferos y de la tierra misma se convierten en basura, desperdicios o residuos (Medina, 1999)

Debido al alto consumismo que existe en la sociedad actual para complacer sus necesidades se genera una alta cantidad de residuos tanto biodegradables y no biodegradables, tal es el caso de los plásticos, principalmente el uso de los polímeros termoplásticos que se emplean en la producción de envases, siendo el polietileno de tereftalato (PET) el producto de uso general de mayor consumo, mismo que se obtiene mediante varios procesos mecánicos como la extrusión, inyección o soplado y el termoformado. Su gran aceptación es porque conserva el aroma y sabor de los alimentos, pero genera una mayor producción de residuos que contribuye a una mayor contaminación ambiental.

La gran parte de países latinoamericanos carecen de políticas y planes nacionales de manejo de residuos sólidos. Bajo estas circunstancias, los gobiernos municipales operan sin guías de política. El manejo de residuos sólidos a menudo consume entre el 20 % y el 40 % de los presupuestos municipales y es un factor importante para las finanzas locales que son subvencionados por el estado. (Medina, 1999)

En el año 2016 en el Ecuador el 34.08 % de los hogares a nivel nacional se dedica a la clasificación de plástico (INEC, 2016), sin embargo, existen muchas personas que se dedican al reciclaje de residuos inorgánicos en botaderos y rellenos sanitarios organizados en asociaciones, una de ellas es la Asociación de Desarrollo Social Romerillos en el cantón Mejía, localizada a 11 km hacia el sur de la cabecera cantonal Machachi, en la quebrada Unión Grande, siendo su acceso a través de la carretera panamericana, con una población actual de 72 miembros directos y 500 miembros indirectos que se desenvuelven actualmente en un área de 11 hectáreas.

El presente estudio tiene como objetivos mejorar las condiciones laborales del personal de la Asociación de Recicladores Romerillos, cantón Mejía-Ecuador, la capacitación del personal, el mantenimiento de equipo existente y el planteamiento de un proceso para dar valor agregado a los ya existentes. Estos objetivos se lograron a través de estrategias del manejo de residuos inorgánicos reciclables acordes a las necesidades, recursos y cultura de la comunidad. Con esta realidad, el Gobierno Autónomo Descentralizado (GAD) de Mejía, realiza un convenio de cooperación con la Universidad de las Fuerzas Armadas -ESPE y la Asociación de Desarrollo Social Romerillos.

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MATERIALES Y MÉTODOS

El presente estudio se realizó en base a una investigación de campo, efectuando diversas visitas técnicas a la planta de Romerillos. Las observaciones están documentadas mediante fotografías y videos, y se pudo constatar las siguientes situaciones:

a) Condiciones laborales. Un aspecto muy sensible que se observó fue el manejo de los residuos inorgánicos, que muchas veces vienen contaminados con desperdicios orgánicos, y que son recuperados por personal de Romerillos de forma manual, autodidacta y sin normas de seguridad, poniendo en riesgo su salud e integridad física, ya que no existe un estudio, valoración y control de los factores de riesgos laborales y mucho menos un manejo técnico de los diferentes tipos de residuos. Este reciclaje es realizado por personas de bajos recursos económicos, adultos mayores y principalmente mujeres que muchas veces acuden con sus hijos pequeños a la planta exponiéndolos de forma directa a muchos factores insalubres propios del lugar, tal como se observa en la Figura 1.

Figura 1. Niño (izquierda) y hombre de la tercera edad (derecha) en el botadero de basura, 2017

b) Estado de la maquinaria. Al realizar varias evaluaciones mecánicas en las diferentes maquinarias disponibles, se verificó que se encuentran obsoletas, sin mantenimiento ni funcionamiento, para lo cual hay que establecer procedimientos para la puesta en marcha, operación y mantenimiento de las mismas. En la Figura 2 se observa el tablero de mando de la prensa hidráulica 1, totalmente descuidado y sin funcionamiento.

Figura 2. Tablero prensa hidráulica 1 sin funcionamiento, 2017.

c) Proceso de clasificación. Es de forma manual ayudado por maquinaria obsoleta. No se posee manual de procesos y procedimientos. En la figura 3, se observa el diagrama de flujo actual del proceso de reciclaje de Romerillos. Cada operación está codificada con un número, por ejemplo, la separación tiene el código 20.

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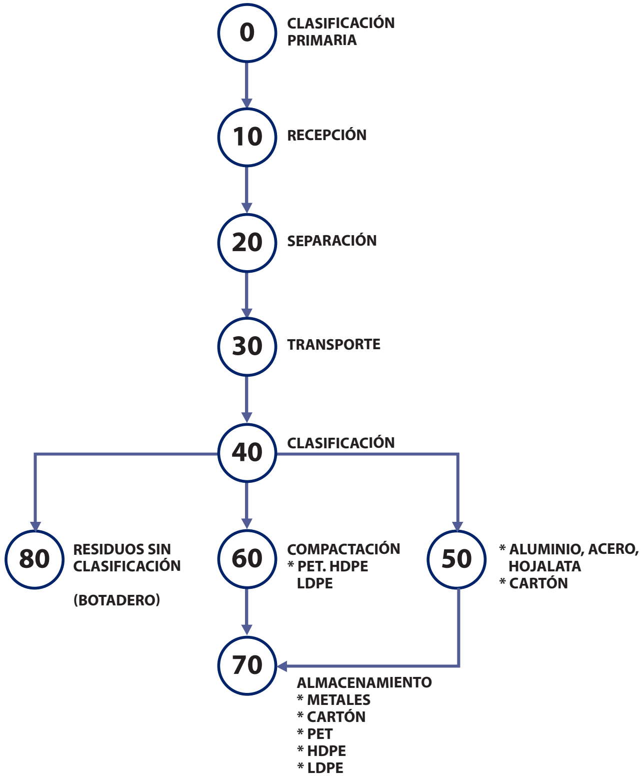

Figura 3. Diagrama de Bloques del Proceso, Centro de Reciclaje "Los Romerillos".

RESULTADOS

Condiciones laborales

En las capacitaciones realizadas hubo un enfoque mayoritario a los principales riesgos en los que se encontraban laborando los recicladores de Romerillos, que son:

- Riesgo biológico: los recicladores de Romerillos están expuestos constantemente a este tipo de riesgos debido a su contacto con los residuos que contienen virus, bacterias, hongos, etc. Además, existen también vectores como roedores, gavilanes, perros y moscas, los cuales son portadores de enfermedades.
- Riesgo Físico: debido a su trabajo a la intemperie, los recicladores de Romerillos soportan cambios de temperatura que oscila entre 4.7 °C y 14 °C al medio día (INAMHI, 2015)
- Riesgo Ergonómico: en el lugar de trabajo de los recicladores no se toma en cuenta las diferencias de género, siendo así que hombres y mujeres cargan bultos en su espalda, cuyo peso sobrepasa los 50 kg, sin protección alguna para llevar de un sitio a otro los productos a clasificar, pudiendo causar lesiones en la espalda, lo que es opuesto a la normativa nacional dispuesta en el Decreto Ejecutivo 2393 (D.E 2393).

Se realizaron capacitaciones en seguridad y salud ocupacional (Figura 4) a todos los miembros de la Asociación Romerillos, con énfasis en el reconocimiento de los riesgos presentes en sus respectivas áreas de trabajo, con especial hincapié en el artículo 11 literal 5 del D.E. 2393, en el cual declara que el empleador debe entregar gratuitamente y en forma obligatoria vestido adecuado y equipos de protección personal (EPP), con el fin de precautelar la salud e integridad física, de esta manera se recomienda el uso principalmente de cascos, guantes, mascarillas, chalecos reflectivos, orejeras, gafas, faja lumbar, zapatos o botas de seguridad y overoles o mandiles de trabajo. También se recomendó el uso de ropa apropiada que garantice las condiciones óptimas de temperatura en las personas, con énfasis en la hidratación y tipo de vestimenta para los meses secos, y ropa de trabajo térmica para la época lluviosa que debería usarse en los meses entre junio y noviembre.

Otra temática cubierta fue la salud ocupacional, específicamente, aspectos de primeros auxilios, tal como se observa en la Figura 5.

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Todas las recomendaciones efectuadas en las capacitaciones, tienen como meta mejorar las condiciones laborales y garantizar su integridad física, psicológica y familiar.

Figura 4. Docentes y estudiantes de la ESPE realizando la capacitación sobre Seguridad Laboral, 2017

Figura 5. Médico de Salud Ocupacional de la ESPE en capacitación, 2017

Estado de la maquinaria

Al realizar las valoraciones mecánicas en las diferentes maquinarias: trituradoras de vidrio, de plástico y de materiales orgánicos; prensa hidráulica, tolva, lavadora de plásticos, banda transportadora, picadora de materiales orgánicos, se observó que se encontraban en pésimo estado de mantenimiento, ante lo cual se implementaron varias soluciones tecnológicas a corto y largo plazo, garantizando su operatividad y disponibilidad, para lo cual se realizaron las siguientes actividades.

- Mantenimiento correctivo: se cambió componentes mecánicos como conjunto de catarinas y cadenas, bandas, ejes, rodamientos, cuchillas de las máquinas picadoras, graseros que faciliten la lubricación de elementos mecánicos internos, válvulas de cierre rápido, y varios componentes eléctricos tales como rebobinaje de motores eléctricos, relés térmicos, luces piloto, botones de accionamiento en tableros de control, breakers, interruptores, tomacorrientes entre otros y finalmente se aplicó pintura. Se entregó la maquinaria funcionando, tras la evaluación, corrección y mejora de los equipos. (Figura 6)

- Lubricación y limpieza de elementos mecánicos sometidos a fricción y desgaste por contacto entre superficies.

- Adecuación de las conexiones de agua, eléctricas e hidráulicas.

- Capacitación al personal en el manejo de la maquinaria para evitar futuras averías y para llevar un control de la programación del mantenimiento preventivo.

- Elaboración de un plan de mantenimiento preventivo y correctivo de las máquinas.

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- Se entregaron manuales de mantenimiento y operación.

- Rotulación a cada máquina y su respectiva botonera de accionamiento.

Figura 6. Máquina clasificadora después del mantenimiento correctivo, 2017

Proceso de clasificación

Conociendo el proceso actual de trabajo que se realiza en el centro de Reciclaje "Romerillos", la Universidad de las Fuerzas Armadas-ESPE a través del grupo de vinculación de la Carrera de Ingeniería Mecánica, sugirió la implementación de un manual de procesos y procedimientos de acuerdo a la disponibilidad de recursos existentes del centro de Reciclaje, además de realizar las siguientes acciones:

- Capacitar a los trabajadores para manejo de materiales y técnicas de etiquetado, según lo dispone la Norma Técnica Ecuatoriana (NTE) 878 del Servicio Ecuatoriano de Normalización (INEN), y sobre procesos de almacenamiento temporal para cada material (TULAS, 2015).

- Asignar un área adecuada para el almacenamiento final de cada material reciclado que no interfiera en la circulación del personal. (NTE INEN 2841).

- Se realizó el levantamiento técnico de la distribución de la maquinaria actual y se realizó una propuesta de mejora.

Propuesta para agregar valor a las actividades realizadas

El proceso actual consiste en la clasificación básica de la materia prima (desechos mezclados) que vienen del botadero de Romerillos. Estos desechos ingresan a la zona de recepción, pasan al tamiz giratorio en el cual se golpea a la materia prima para forzar la separación en productos individuales, luego van a la banda transportadora donde se clasifican en estaciones específicas de cada producto. Finalmente, algunos productos son compactados y terminan en el área de almacenamiento tal como lo indica la legislación ecuatoriana en la Reforma del Tulas Libro VI artículo 63, en el parágrafo III del Almacenamiento Temporal. Por lo descrito, se observó que el reciclaje en la planta de Romerillos, posee procesos establecidos que generan poco valor agregado, además de carecer de una conducción técnica y organizada de los procesos.

La recolección en todo el cantón bordea las 59 ton/día de desechos sólidos, mientras que en las parroquias se recolecta 45 toneladas/día, recuperando 2.65 ton/día de desechos inorgánicos gracias a la Asociación de Desarrollo Social Romerillos y en desechos orgánico se recupera alrededor de 0.125 ton/día. (Dirección de Servicios Públicos e Higiene del Cantón Mejía, 2014). Según los datos estadísticos recogidos en los años 2016 y 2017, se recolectaron las cantidades de plástico detalladas en el Tabla1.

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Tabla 1

Ingresos y ventas anuales 2016 - 2017 de la Asociación de Desarrollo Social Romerillos

Material		AÑO 2016				AÑO 2017			
	Valor Kg	Total		Valores ($)		Total		Valores ($)	
	$Kg	Kg	$	Depositados Municipio	A Romerillos	Kg	$	Depositados Municipio	A Romerillos
Pet de cola Embalado	0,70	25590,00	17913,00	255,90	17657,10	21770,00	15239,00	217,70	15021,30
Pet duro	0,30	21540,00	6462,00	215,40	6246,60	17220,00	5166,00	172,20	4993,80
Pet Soplado	0,20	31080,00	6216,00	310,80	5905,20	25180,00	5036,00	251,80	4784,20
Plástico de alta y baja	0,15	101680,00	15252,00	1016,80	14235,20	77470,00	11620,50	774,70	10845,80
Zapatillas	0,15	2980,00	447,00	29,80	417,20	1500,00	225,00	15,00	210,00
Total		182870,00	46290,00	1828,70	44461,30	143140,00	37286,50	1431,40	35855,10

Fuente: Asociación Romerillos, 2017

La Asociación de Desarrollo Social Romerillos desea adquirir habilidades y destrezas para el procesamiento de plástico reciclado siguiendo la recomendación del artículo 73 de la Reforma Tulas Libro VI de la legislación ecuatoriana, para lo cual proponen la implementación de un proceso de elaboración de postes ecológicos para cercas o cerramientos ganaderos.

De esta manera el grupo de vinculación con la sociedad de la Carrera de Ingeniería Mecánica de la Universidad de las Fuerzas Armadas del Ecuador-ESPE, desarrollaron el proceso de la elaboración el producto solicitado por los recicladores de Romerillos.

Según la revista digital Ambientum 2003, en el mercado internacional existen diversos productos que se elaboran con materiales provenientes del reciclado en un 100 %, así como de combinaciones con plásticos vírgenes al 75 %, 50 %, etc. En un estudio presentado por Guerrón & Juiña, 2013, en base a una mezcla de materiales reciclados conformada en un 90 % por PEAD y 10 % de PEBD, obtuvieron una combinación óptima para la elaboración de ecopostes. Medina, 1999, señala que entre las principales características que diferencian a la madera de los postes plásticos son:

- Durabilidad cinco veces mayor que la madera natural
- No necesita pintura, tintes especiales, ni selladores para alargar su vida útil
- Resistente al agua, la humedad y los solventes químicos
- Contribuye al mejoramiento del ambiente
- Ayuda a conservar el área forestal, reduciendo la depredación de los bosques
- Ayuda a reducir los desechos sólidos, transformándolos en productos útiles
- No absorbe ni almacena agentes fisio-sanitarios, bacterias, hongos o plagas
- Inmune a microorganismos como los insectos y roedores
- No necesita mantenimiento
- No se raja ni se astilla.

Dada la experiencia de producción en el mercado local de parte de varias industrias productoras de ecopostes y de acuerdo con el

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pedido de la Asociación Romerillos, se plantea producir un 70 % con un peso de 6 kg y un 30 % de 8 kg. Los ecopostes a producir con plásticos de baja y alta densidad pueden adoptar varias formas para cubrir cualquier necesidad debido a las favorables propiedades dúctiles que tiene el plástico, de esta manera se propone elaborar ecopostes que poseen principalmente las dimensiones descritas en la Tabla 2, además se busca contribuir a la conservación de los recursos naturales principalmente evitando la deforestación de los bosques con la producción de dichos postes ecológicos.

Tabla 2

Especificaciones del producto

Dimensiones	Unidad	ECOPOSTE	
		6 kg	8 kg
Sección transversal (b)	mm	70	80
Sección transversal (h)	mm	70	80
Largo	mm	1800	1800
Volumen	cm^3	8820	11520
Densidad	kg/cm^3	0,074	0,074
Peso kg	kg	6,53	8,52
Eficiencia proceso	! "	90	90
Peso materia prima	kg	7,25	9,47

Se realiza un estudio del proceso para la elaboración de los ecopostes en base al cálculo de materia prima necesaria para cubrir la demanda impuesta de 7000 ecopostes/mes, como se presenta en la Tabla 3.

Tabla 3

Proyección de producción de ecopostes para el año 2020

TIPO	PESO		% Producción	Materia Prima	Producción mensual
	Poste (Kg)	MP (Kg)		Kg/mes	u/mes
Ecoposte 8	8,52	9,47	30	19891	2100
Ecoposte 6	6,53	7,25	70	35535	4900
TOTAL				55426	7000

El proceso de elaboración de los ecopostes se plantea desarrollarlo en varias etapas como se indica en la Figura 7. Una ventaja de este proceso es que se empleará mayoritariamente la maquinaria existente en la planta de reciclaje de la Cooperativa Romerillos.

El proceso iniciará con una clasificación del material reciclado con el fin de obtener PEAD y PEBD, posteriormente se realizará el triturado hasta conseguir pequeños gránulos los mismos que serán lavados y secados. Posteriormente irán hacia la extrusora que posee un dado extrusor el cual dará la forma de la sección que tendrá el ecoposte requerido. Para conseguir esta última etapa del proceso es necesaria la adquisición de una extrusora, que cubra la capacidad de producción requerida según la proyección estimada de materia prima recolectada que bordea los 55426 kg/mes y procesando cerca de 200 a 400 kg/h durante una jornada de trabajo de 8 horas.

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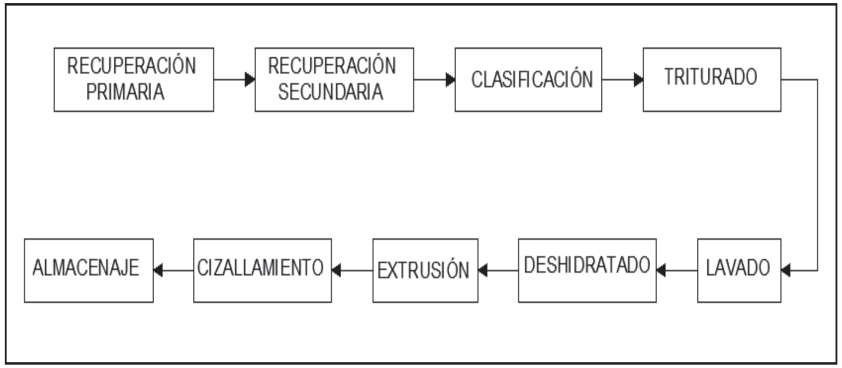

Figura 7. Proceso de producción de Ecopostes

DISCUSIÓN DE RESULTADOS

Como se evidenció en el desarrollo de este trabajo, las condiciones de trabajo de los recicladores, en general, son deficientes. La sociedad no valora al reciclaje en su verdadera dimensión y como menciona Torres, 2012, el reciclaje no es sólo el proceso por el cual se amplía el ciclo de vida de los materiales y se optimizan los recursos, sino que también es un negocio, una industria, por lo que los recicladores no son seres "desechables" sino personas que con su oficio le dan vida al planeta. Por su actividad, los recicladores van por las calles buscando entre basuras material útil, teniendo constante contacto con materiales insalubres y vapores tóxicos lo que representa un alto riesgo de enfermedad. Pueden también sufrir accidentes al herirse con materiales cortopunzantes y lesiones lumbares por transportar el material durante largas jornadas de trabajo. La falta de capacitación agrava este tipo de accidentes pues no tienen formación sobre el manejo de residuos. Todo esto se constata en las condiciones en las que laboraban los trabajadores de la Asociación de recicladores Romerillos. La capacitación impartida referente a los diferentes tipos de riesgo pretende disminuir los accidentes y las enfermedades laborales en este grupo vulnerable de la sociedad.

Optimizar las condiciones laborales de los recicladores, también conlleva mejorar la maquinaria y los procesos productivos. Es por ello que los estudiantes y docentes de la Carrera de Ingeniería Mecánica de la Universidad de las Fuerzas Armadas-ESPE, aportaron con su contingente de conocimientos, económicos y sociales para dejar la maquinaria en óptimo estado. Mostrar otra opción para mejorar los ingresos de cada uno de los integrantes de la Asociación de Recicladores de Desarrollo Social Romerillos fue la mayor contribución para subir los 300 usd mensuales que reciben trabajando jornadas de 12 y 14 horas por día.

En este estudio no se ha tomado en cuenta a factores políticos y sociales que deben ser encabezados por los diferentes gobiernos seccionales para el mejoramiento de la calidad de vida de esta población por lo que sería viable que se complemente con un estudio en esa línea.

CONCLUSIONES

Se desarrolló el proceso para agregar valor al actual: elaboración de ecopostes, solicitado por los recicladores de Romerillos, a través del mismo se estableció un manejo y cantidades adecuadas de la materia prima necesaria, secuencia de los procesos y maquinaria necesaria.

Los estudiantes y docentes de la Carrera de Ingeniería Mecánica de la Universidad de las Fuerzas Armadas ESPE, a través de la ejecución del proyecto de vinculación con la sociedad "Mejoramiento de condiciones laborales del personal de la Asociación de Recicladores Romerillos" contribuyeron con su conocimiento, su trabajo y compromiso social a la solución de un problema. A su vez, los estudiantes tuvieron la oportunidad de fortalecer su formación técnica y humanista, para con esta visión enfrentar, a futuro, los problemas del mundo laboral.

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Assessing the penetration of bioethanol in the andean community: a review

Evaluación de la penetración del bioetanol en la comunidad andina: una revisión

*ANA BELÉN GUERRERO[a,b], MARÍA DOLORES CURT[a]

[a]Departamento de Producción Agraria, Universidad Politécnica de Madrid (UPM). Madrid, Spain
[b]Departamento de Sostenibilidad. Trisquel Consulting Group. Quito, Ecuador
*ana.guerrero@trisquel.ec

ABSTRACT

The sustainable development agenda of the United Nations aims to ensure affordable energy for all and to diminish greenhouse gas emissions in order to mitigate the effects of climate change. A way to achieve these goals is by the substitution of fossil fuels with biofuels. This study compares three Andean countries: Colombia, Ecuador and Peru, in terms of bioethanol blending mandates current scenario and its sustainability. For this analysis, a review of the state of the art of first and second generation bioethanol in the three countries were developed, including a social network analysis to understand the interactions that have enhanced or delayed the achievement of their blending objectives as countries. So far, Colombia is near to reach its blending target; Ecuador has recorded a bioethanol deficit, estimated at 75%; and regarding Peru, in the last years they have achieved their national target. In addition to blending mandates, the countries appear not to rely on a sustainability policy for the development of first or second generation bioethanol. Furthermore, these Andean countries are planning to increase their blending targets. In order to achieve this, political efforts must be focused on feedstock availability as well as on the development of guidelines related to sustainability and technologies to be applied in their own context.

Keywords:

Policy, Latin America, sustainable development, first and second generation ethanol.

INTRODUCTION

Climate change is the most economically and politically complex problem that humanity is currently facing (Sachs, 2015). For this reason, climate change is at the top of the sustainable agenda, and minimizing its impact on the environment has turned as a priority in the post 2015 development agenda, where developing countries are expected to play an increasing role in global climate change mitigation.

In 2015, the United Nations (UN) proved that the global emissions of carbon dioxide (CO2) continued their upward trend (United

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Nations, 2015). In fact, on a global average basis, historical levels of CO2 were reached in this year (400 ppm for the first time) (WMO, 2016). Hence, the UN established the seventh goal of the Sustainable Development Goals (SDG) in the post 2015 agenda -which aim is "to ensure access to affordable, reliable, sustainable and modern energy for all"- for which countries have to bet for renewables to diminish climate change impact, but countries commitment is needed. In fact, since the Conference of the Parties (COP21), 175 out of 197 parties have ratified the Paris Agreement. The central aim of the agreement is to strengthen the global response to the threat of climate change by keeping a global temperature below 2 °C above pre-industrial levels (UNFCCC, 2018).

Facing the problems derived from climate change and the increasing scarcity of petroleum resources, many nations are turning to renewable energy. There is a wide range of renewable technologies (bioenergy, solar, wind, geothermal and others) that can be used depending on the availability of natural resources in each country, but biomass receives priority because it is the single largest renewable energy source that can be directly used for production of biofuels (Dwivedi, Alavalapati, & Lal, 2009; IEA, 2015).

Considering energy as an indispensable component of society, and transportation the activity that demands the largest share of final energy in the world (Ballesteros, 2010a), which implies half of the world oil consumption and a fifth of its greenhouse gas (GHG) emissions (IRENA, 2016), the scientific community and global leaders have taken responsibility to maintain energy security and enhance the development of sustainable, clean and affordable transportation fuels in order to achieve the SDG (B. Solomon & Bailis, 2014). It means that governments must play a hinge role, making societal needs a priority and promoting research on renewables in order to improve the wellbeing of the society (Figure 1).

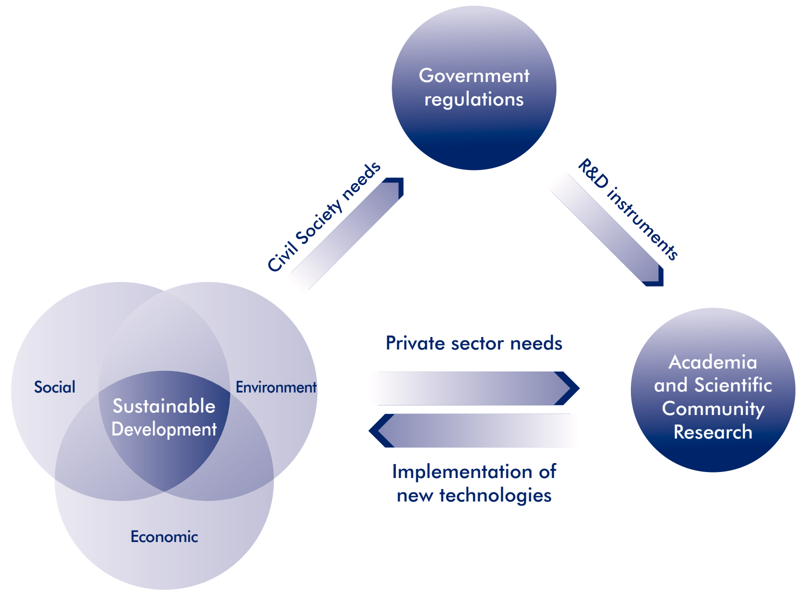

Figure 1. Relation and role of the government, scientific community and civil society to achieve a sustainable development.

Liquid biofuels can contribute to guarantee energy security and diminish the current fossil fuel dependence. In addition, biofuels have the potential to improve air quality and mitigate global warming on the grounds of a reduction in GHG emissions. Furthermore, biofuel production can provide new income and employment opportunities in urban and rural areas. Indeed, in a global overview developed by the Renewable Energy Policy Network for the 21st Century (REN21), in 2015 liquid biofuels were positioned as the second largest employer in the renewables area with 1.6 million jobs (REN21, 2016).

Biodiesel and bioethanol are the main liquid biofuels in the market. This study focuses on bioethanol since it is the most widely used biofuel worldwide (Ballesteros, 2010b). There is no generic

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agreement for international standard classification of bioethanol; however, three main categories of biofuels are usually recognized: (i) First generation (1G), which is produced mainly from food-based feedstock or staple crops of high saccharide content like sugarcane, maize and wheat, and has already reached a commercial stage. (ii) Second generation (2G), which is produced from non-edible biomass or lignocellulosic plant materials, like forestry and agricultural residues and municipal solid wastes, and involves cheap and abundant feedstock (Naik, Goud, Rout, & Dalai, 2010), but an intermediate technology is needed for its biochemical or thermochemical conversion (Strezov, 2014). Lignocellulosic biomass has gained attention as being a potential low-cost feedstock that avoids competition with food demand, especially the use of forest and agricultural residues (Takara, Shrestha, & Khanal, 2010). (iii) Third generation (3G) ethanol, currently in laboratory experimentation with the use of algae and genetically modified organisms (Strezov, 2014).

Nowadays, Brazil and the United States have a well-established 1G bioethanol industries, and lead the ethanol production of the world representing 30% and 57%, respectively, of the worldwide production (98.3 billion liters) (REN21, 2016). US uses as main feedstock maize and Brazil sugarcane. However, concerns about the sustainability of this type of biofuel, which is based on edible crops as feedstock, have been raised (B. D. Solomon, Banerjee, Acevedo, Halvorsen, & Eastmond, 2014) due to a possible food vs. fuel market competition, direct and indirect land-use changes, and biodiversity loss.

Some developing countries are also interested in bioethanol production to encourage rural development, diminish fossil fuel importations and diminish GHG emissions in furtherance of the SDG compliance. According to Pistonesi et al. (2008) the production of biofuels in Latin America could be seen as a strategy to achieve agricultural, environmental and energy development. Specifically, South America is considered as a territory that has large renewable energy resources, which could contribute to the coverage of the world's energy demands (Janssen & Rutz, 2011).

The purpose of this article is to review the state of the art of 1G bioethanol production in three countries of the Andean Community: Colombia, Ecuador and Peru, compare the regulations established to promote bioethanol production, and determine if these countries have tools to ensure the sustainable development of biofuels. These three countries were chosen because they are neighboring countries, possess extensive land areas with similar climate conditions, and in consequence cultivate similar crops and produce similar agricultural residues. Additionally, they exhibit different stages of maturity of their political agenda regarding biofuels. To the best of our knowledge, developing countries that have demonstrated interest in biofuels in the last decade have been barely considered in research or report works, but an in-depth study is needed to understand the implications of the success or delay of the progress of biofuels in these countries.

METHODS

Collection of information

To analyze blending mandates and legal framework that leads the development of the bioethanol industry in the countries under study, all the legal instruments related to biofuels were studied and are listed in Table 1. An analysis of sustainability instruments used in each country to ensure the sustained development of bioethanol in the social, environmental and economic aspects was developed. This includes policies analyses that reinforce the government duty in each legal framework, and the study of some multilateral reports.

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Social Network Analysis

The legal framework related to the enhancement of biofuels in the three countries was analyzed and the main actors were detected (listed below), including the responsibilities and duties that the law assigned. Then, a social network analysis was developed using UCINET 6 and NetDraw software (Analytic Technologies, US) following the methodology described in (Moncayo Miño & Yagüe Blanco, 2016). Social network analysis allows creating a network map on interaction among stakeholders of a sector (Vantoch-Wood & Connor, 2013). It provides a way of making tangible some areas where the political will has incidence and its interactions. In addition, some effects can be studied like communication network structure (Borgatti, Mahra, Brass, & Labianca, 2009), network weakness, gaps and patterns (Vantoch-Wood & Connor, 2013). Network research has been widely used in a great number of fields, including social sciences, physics and biology. In addition, several applied fields use this tool such as governance (Bonvecchi, Johannsen, Morales, & Scartascini, 2015; Borg, Toikka, & Primmer, 2015), public policy (Ingold, 2014) and natural resources (Bodin & Crona, 2009).

For the analysis, six different areas of incidence of the law were considered: social, economic, environmental, research & development (R&D), productive and regulatory. These areas were considered for the analysis because they are prioritized by the laws analyzed in Table 1, and they are under the umbrella of biofuels purposes and its sustainable development. Betweenness centrality was selected as the analyzed variable. This measure represents the number of times that a node is in the way of two nodes that are not related (Wasserman & Faust, 1994). However, the approach for a network analysis on selected laws and the visualization of connection between areas of incidence is a procedure rooted in mathematical and physical domains of network theory (Newman, 2003).

Table 1

Legal framework related to the enhance of bioethanol industry and blending mandates

Year	Colombia	Ecuador	Peru
2001	Law 693	-	-
2002	Law 788	-	-
2003	Resolution 180687	-	Law 28054
2004	Law 939	-	-
2005	-	-	Supreme Decree 013
2006	-	-	Resolution 400
2007	Decree 2629	-	Supreme Decree 021, Resolution 014
2008	CONPES 3510	-	-
2009	-	Executive Decree 1831, Executive Decree 1879	Supreme Decree 091, Resolution 206, Resolution 515
2010	-	Organic Law of Food Sovereignty	Supreme Decree 061
2011	Decree 4892	Executive Decree 971	Supreme Decree 024
2013	Resolution 90932	Executive Decree 1048	-
2015	Resolution 41072	Executive Decree 799, Executive Decree 675, Resolution 031	-
2016	Resolution 789	-	-

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In the Colombian case, 11 main actors were identified: Ministry of Mines and Energy (MME), Ministry of Agriculture and Rural Development (MADR), Ministry of Transportation (MT), Ministry of Environment and Sustainable Development (MADS), National Planning Department (DNP), Ministry of Finance and Public Credit (MHCP), Ministry of Commerce, Industry and Tourism (MCIT), Administrative Department of Science, Technology and Innovation (COLCIENCIAS), Ministry of Health and Social Protection (MSPS), Banking Superintendence (SB), and Superintendence of Ports and Transports (SPT).

For Ecuador, nine actors were considered as key players for the development of biofuels according to the law: the Central Government (CG), Ministry of Agriculture, Livestock, Aquaculture and Fisheries (MAGAP), Coordinating Ministry of Production, Employment and Competitiveness (MCPEC), Ecuadorian Standardization Service (INEN), Public Company of Hydrocarbons of Ecuador (EP PETROECUADOR), Regulatory and Control Agency Of Hydrocarbons (ARCH), National Institute of Energy Efficiency and Renewable Energies (INER), National Customs Service of Ecuador (SENAE), and Coordinating Ministry of Strategic Sectors (MICSE). It is important to highlight that since 2017, some of these institutions have disappeared due to the change of government and certain competences have not yet been granted to other institutions.

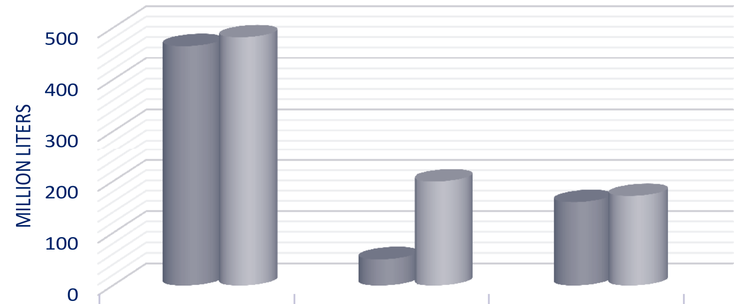
Finally, in the Peruvian case, 12 institutions were recognized as main actors related to the development and enhancement of biofuels intended for ground transport: National Commission for Development and Life without Drugs (DEVIDA), Agency for the Promotion of Private Investment (PROINVERSION), Ministry of Energy and Mines (MINEM), National Environment Council (CONAM), Petróleos del Perú (PETROPERÚ), Ministry of Economy and Finance (MEF), Supervisory Body of Investment in Energy and Mining (OSINERGMIN), Ministry of Production (PRODUCE), Ministry of Agriculture and Irrigation (MINAG), National Council of Science, Technology and Technological Innovation (CONCYTEC), Selva y Sierra Exportadora (S&S Exportadora), National Institute for the Defense of Competition and Protection of Intellectual Property (INDECOPI).

RESULTS AND DISCUSSION

Biofuel targets and blending mandates

Biofuel blending mandates are one of the most common policies used in the transportation sector that enhance the use of renewable energy (UNCTAD, 2016). Government policies have played a major role in the development and expansion of the biofuel industry globally over the last decades. In this sense, some countries from Latin America have developed policies to promote biofuels production based on successful experiences like in the US and Brazil (Pérez-Peña & Acharya, 2015).

Biofuel mandates and targets have been established in 66 countries, of which 19 are in the Americas, including the countries under study: Colombia, Ecuador and Peru (Lane, 2018; Maltsoglou, Koizumi, & Felix, 2013).



	Colombia	Ecuador	Peru
Production	465	50	160
Consumption	482	200	172

Source: Colombia 2016 (USDA, 2016), Ecuador 2016 (Ministerio de Producción, 2016), and Peru 2015 (OECD/FAO, 2014).

Figure 2. Relation between production and consumption of bioethanol in Colombia, Ecuador and Peru.

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Colombia

Colombia has emerged as one of the largest producers of biofuels in Latin America, and currently is producing both oil palm biodiesel and sugarcane bioethanol. The bioethanol mandate stipulates a national use of E8 and E10 (10% anhydrous ethanol and 90% gasoline) for ground transportation depending on the region (Global Renewable Fuels Alliance, 2015).

The blending mandate is accompanied by tax incentives for selling bioethanol and importing machinery. Ethanol prices are fixed by the government based on a monthly calculated price, where a parity price for international sugar prices is applied (USDA, 2016). Despite the establishment of legal instruments to encourage the development of the biofuels industry, Colombia has not covered its national demand (see Figure 2), and ethanol from other countries is imported.

Figure 3a shows that the efforts to promote the use of biofuels in Colombia are focused on the economic area, where more actors are involved and more actions have been devoted. Moreover, the network shows that the Ministry of Mines and Energy (MME) and the Ministry of Agriculture and Rural Development (MADR) are the key players because they share responsibilities in more areas of incidence than other actors, being their centrality measures higher than the others are. Indeed, the network analysis shows a centralized structure in a form of star, which is considered as the fastest performing network (Borgatti et al., 2009). The net shows connection between their actors, including five of the six areas studied. In consequence, their joint efforts allow the development of biofuels in the country, but the social aspect must be improved. The implementation of biofuel laws requires a holistic approach in order to ensure its sustainability.

Although Colombia is near to achieve their blending target, ethanol producers seem not to have security to continue investing. Indeed, there was the need to seek government protection because of high ethanol imports from the United States, which hit record levels in 2013 due to the preferences granted in the Trade Promotion Agreement set between both countries. In 2014, the Colombian government had to restrict ethanol imports, only allowing when the domestic supplies run out of anhydrous ethanol for its blending mandate fulfillment (USDA, 2016).

Colombia shows a trend to a gradual increase of biofuels aiming at the partial substitution of fossil fuels and reduction on importation of energy (Cremonez et al., 2015). Indeed, the government aims to increase ethanol blends up to 25% by 2020 (Sorda, Banse, & Kemfert, 2010), but according to our approach, there is no public policy that ensures this. Colombia's ethanol supply is safeguarded due to the possibility of imports in the case of the lack of ethanol in the country, but the private industry has no security on their long-term production due to the government's lack of clarity on the issue that blending mandates will increase over time.

It is worth mentioning that Colombia clearly presents a more developed industry in the private sector, which has already reach the organizational stage of association named "Fedebiocombustibles", since 2004. This association has become an active actor in the promotion and strengthening in biofuels projects.

Ecuador

Ecuador's Constitution encourages the development of renewable energy sources. In 2004 the government declared biofuels as a "national interest" by executive decree, but there was a lack of specific legislation and regulations for biofuels development and production (USDA, 2012) and later that decree was repealed. After this, it was not until 2010 that the country started activities related to biofuels. A pilot

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program named Ecopais started in that year, which main aim was to test the consumer acceptance of an E5 gasoline. During the time of the pilot program, the government regulated the price of ethanol at USD 0.76/L, and such program gave purchase priority to artisanal ethanol producers. However, since May 2015, the price has been set according to the Argus US Gulf Coast ethanol price, and the government ensured that the price payed to the producers could not be less than USD 0.90/L (bottom band).

The pilot program was a success, so in 2014 the government stablished the mandate to achieve the full replacement of common gasoline with E5 blended gasoline in 2017, followed by full E10 blend in 2018. However, this target is a challenge because the production in 2015, which was entirely from sugarcane, only covered 25% of the national demand, as it is shown in Figure 2, and until December 2017, E5 blended gasoline was distributed in eight of the 24 provinces of Ecuador (EPPetroecuador, 2017). This fact demonstrates that the national mandate was very ambitious in quantity and time of execution. Though some efforts have been done, it have not been enough considering that during 2017 some of the institutions responsible for the execution were suppressed, causing instability in the ethanol production.

Figure 3b clearly shows many structural holes in the network because it illustrates an open binding mechanism, and it is the network with more excluded nodes demonstrating weakness. In fact, the network shows disconnection in three of the six studied areas. When a network present many structural holes demonstrate disconnection between actors. This effect could be one explanation of the delay observed in Ecuador compared to its neighboring countries. In addition, the net shows that the efforts of the government, in legal matter, were directed towards strengthening the productive and economic areas, since the main objective of the ecopais program was to decrease imports of high-octane naphtha used in the preparation of common gasoline.

Considering that biofuels by concept are intended to reduce GHG emissions in order to mitigate the effects of climate change, no law has considered the Ministry of Environment as an actor, to make sure that the development of biofuels in the country is supported with environmental criteria. Moreover, as key player stands out the Coordinating Ministry of Production, Employment and Competitiveness, to whom all the competences related to biofuels were transferred and centralized. This is a major weakness because if this actor does not accomplish its duties, recognized by the law, the interconnection between regulatory, economic and productive areas could fail. Nowadays, this ministry does not exist.

Ecuador has a clear potential to establish a biofuel industry because of its existing biomass resources, geographical advantages, and its high level of interest. In addition, the country is clearly aware that the food vs. fuel competition has to be avoided. Ecuador is one of the seven countries of Latin America that prioritize food security over biofuels (Bailis, Solomon, Moser, & Hildebrandt, 2014). In fact, the Organic Law of Food Sovereignty (2010) states the prioritization of food production "as long as possible" over biofuels production (IRENA, 2015). Thus, Ecuador should search for more or new feedstock for bioethanol production because the country is highly dedicated to agriculture and agroindustry (9.4% of the gross domestic product - GDP) (The World Bank, 2014).

To achieve Ecuador's mandate while the bioethanol industry turns auto-sustainable, the government must start granting the competences of the disappeared MCPEC to another institution that is able to create and enhance alliances among the productive sector of biomass, processing industry, retailers and oil companies.

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General challenges comprise the mismatch between bioethanol processing capacities and the feedstock supply sector, and/or the risk of insufficient raw material. Thus, a milestone that must be included in the political agenda is to reach the installed bioethanol processing capacity (Maltsoglou et al., 2013). The achievement of the mandates must go hand in hand with careful planning in order to avoid the dependence of biofuel importation.

Peru

In 2003, the country adopted policies to enhance biofuel production. Afterwards, in 2007 the government established target E7.8, which is obligatory in a national scale since 2010 (Cremonez et al., 2015). The blending mandate was expected to contribute to energy security, encourage investments, promote rural and socioeconomic development, diversify the agricultural sector, create employment opportunities and protect the environment. Peruvian policy makers explicitly stipulated the objective of biofuel production as an alternative to the illicit cultivation of coca leaves contributing to the National Strategy to Combat Drugs (B. Solomon & Bailis, 2014).

Since 2011, Peru has accomplished its blending target with a whole production of bioethanol from sugarcane (USDA, 2015), but in 2015 the country struggled to cover the national demand (see Figure 2) because of the closure of the main ethanol plants. Peru is one of the smaller producers of biofuels in Latin America and the Caribbean, and its production is mostly from sugarcane, but the government is also considering promoting the use of molasses. (B. Solomon & Bailis, 2014).

Peru reached its bioethanol autonomy in a relatively short period since the country is committed to fulfilling its blending targets. Figure 3c shows that Peru has devoted its efforts to work articulately. This network demonstrates many interactions and high political will. It means that actors require coordination mechanisms in order to achieve a fluently acting. The government has been enhancing the productive and R&D areas of biofuels, but the environmental incidence must be improved. Although the Ministry of Environment (CONAM) is considered as an actor in the normativity, no environmental regulations have been devoted to ensure the environmental sustainability of bioethanol. The key players are the Ministry of Energy and Mines, and PETROPERÚ, which is interesting because in this way Peru benefited of the installed capacity of the main institutions related with oil in order to make a smooth transition to this new business turnaround, benefiting of the know how that they already had about hydrocarbons.

As discussed, the three countries have political will and blending mandates, but each country has a different bioethanol industry development level. Colombia and Peru demonstrated interest for biofuels at the beginning of the 2000s and seventeen years later, they are near to achieve or have already achieved their national blending targets. This fact is also reflected in the betweenness centrality (Table 2) where Colombia and Peru have more incidence areas with values >0. Betweenness centrality takes value 0 when an incidence area has interaction with one actor or no interaction, and 1 when a node interacts with all the actors of the net. Meanwhile, Ecuador is delayed in the achievement of its national mandates and it could be considered that is at an early stage. Precisely for this reason, this network does not present the same maturity as the other two countries. It means that its betweenness centrality (Table 2) is 0 in four of the six areas and could be motivated by the recent interest in biofuels of the country. In addition, Colombia and Peru exhibit laws that enhance and sustain biofuels, and Ecuador has executive decrees, which turns biofuels in a vulnerable initiative to future political changes such as the close of some ministries as it has been reported previously. The three countries have one area of incidence that have not considered in their legal frameworks. In Colombia, the social incidence needs to be strengthened, and in Ecuador and Peru,

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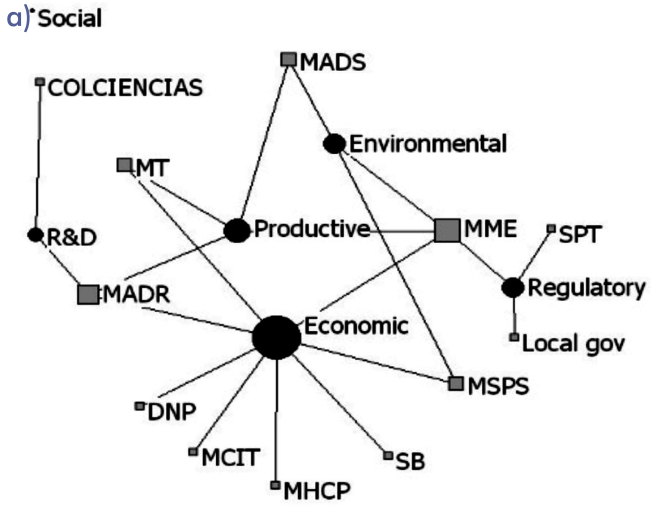
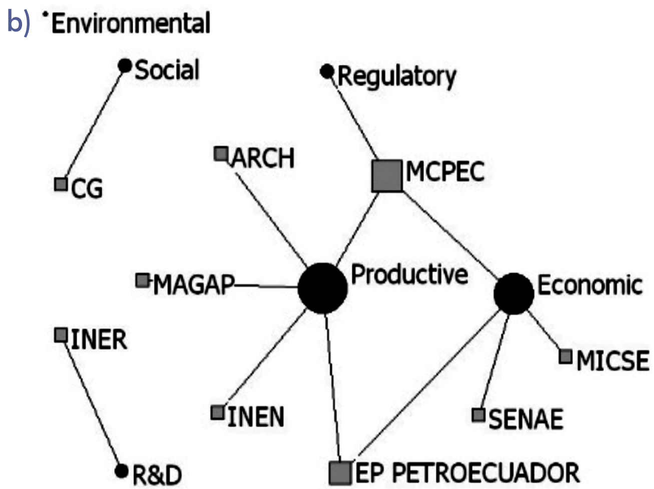
the environmental area needs to be considered in order to ensure the environmental sustainability of bioethanol production.





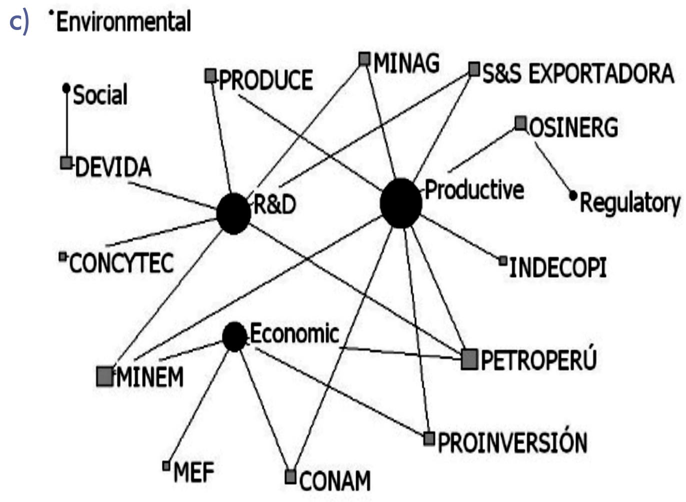


Figure 3. Social network analysis a) Colombia, b) Ecuador, and c) Peru. Circular nodes represent incidence areas, and square nodes represent actors identified in the laws. Each relation between actors and areas is represented by a line. The size of the nodes differ according to the betweenness centrality.

Table 2

Betweenes centrality in the incidence areas studied in Colombia, Ecuador and Peru. Data normalized.

	Colombia	Ecuador	Peru
Environmental	0.06	0.00	0.00
Social	0.00	0.00	0.00
Economic	0.55	0.19	0.15
Productive	0.16	0.26	0.49
i+D	0.11	0.00	0.37
Regulatory	0.22	0.00	0.00

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Sustainability

Bioethanol production has benefits and impacts on social, economic and environmental areas that vary from country to country because each one has different feedstocks, yields, transformation technologies and level of development that better suit to each national context. It is a mistake to judge biofuels in a generic way because the possibilities to shift to biofuels and the objectives to be achieved are different in each country, including the political will (Escobar et al., 2009).

One of the advantages of bioethanol lies in its local production, which is translated into a reduction of GHG emissions in feedstock transportation, the maintenance of rural life and its economic development. In fact, Brazil is a successful case in covering these three aspects. Since the 1970s, sugarcane ethanol industry in Brazil has reinvigorated 10% of Brazilian population from poverty (Nanda, Azargohar, Dalai, & Kozinski, 2015). Moreover, Brazil overcame the necessity of subsidies for that sector and now it is economically sustainable. Indeed, foreign investors are increasing rapidly in the country due to the extraordinary capacity of ethanol production, which has been reflected in the increase of job opportunities (Hira & de Oliveira, 2009).

Considering the fundamentals of biofuels and its dependency to a country context, sustainability has to be addressed by each government, developing policies that guarantee the three dimensions of sustainability.

As demonstrated in "Biofuel targets and blending mandates" section, Colombia, Ecuador and Peru have not considered one of the three areas of sustainability in their legal framework. Some multilateral biofuel certification systems for sustainability, and technical instruments developed by non-governmental organizations (B. D. Solomon et al., 2014) could be implemented in these countries in order to ensure the sustainability of bioethanol.

On an international level, the Global Bioenergy Partnership (GBEP) coordinated an agreement on a list of 24 sustainability indicators to guide national efforts in bioenergy sector development. These indicators are voluntary and cover socioeconomic and environmental sustainability (B. Solomon & Bailis, 2014). Colombia was chosen as the Latin American country to pilot test the GBEP sustainability indicators, and counts with a study developed with life cycle assessment approach devoted to ensure the environmental sustainability of biofuels (Consorcio CUE, 2012).

The 24 indicators by GBEP can serve to guide the countries to develop their own indicators focusing on the base line to determine the objectives that must be achieved. These should be developed with technical and scientific criteria and be based on the opinion of stakeholders, in order to decrease the gap between science and policies and to support a reflection on the decision-making process (Gomes, Malheiros, Fernandes, & Maria, 2015). Some studies that analyzed the environmental and social effects of biofuels in developing countries recognize that biofuels sustainability depends on natural conditions, socio-economic setting and feedstock production systems, which implies that the impact of biofuels may vary from country to country (van Eijck, Batidzirai, & Faaij, 2014).

The challenge of improving the feedstock supply through yield improvement and feedstock diversity in more sustainable ways can be accomplished, but only with prolonged support and sensible, easily adoptable policies that recognize the environmental as well as the economic objectives (UNCTAD, 2016). Deciding on energy future is not only a matter of responsibility of technology developers, funders and users; the most important responsibility falls in the political commitment

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to more participatory, comprehensive and transparent practices in the appraisal of technological change (Ribeiro & Quintanilla, 2015). More investments in R&D programs are required so that technologies for biofuel production could fit the needs of the countries (Escobar et al., 2009).

CONCLUSIONS

The three countries studied have bioethanol blending mandates. Colombia has not reached its blending target falling short by 3.5%; Ecuador has recorded a large bioethanol deficit, estimated at 75% for 2016, whereas Peru has already achieved its national target.

Research and innovation on new conversion technologies, feedstocks and economics of liquid biofuels are available in the studied countries, but governments have an obligation to use all possible instruments to promote R&D with public funds to enhance the development of biofuels technology.

To ensure bioethanol industry development and sustainability, biofuel policy must be based on an exhaustive analysis of the country's context, including food security, feedstocks and bioenergy potential. The technology is there, but a supportive legislation with a clear mandate is necessary. It will be a challenge for policy makers and industry executives to improve the continued expansion of the biofuel sector, using available tools and to develop new ones in order to ensure its sustainability.

Concerning the network approach used in this study, it is important to highlight that in some cases what is stated in the law is different to what is actually happening in reality. Nevertheless, this review could provide direction for decision makers and scientific community towards relegated topics and stimulate greater integration of the network.

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Production of lipids from psychrophilic microalgae present in antarctic glaciers for the synthesis of biofuel.

HUILCA,G[a]; LICTO,L[b]; FLORES,R[c]; PROAÑO,F.[a]*

[a]Department of Life Sciences, Faculty of Biotechnology, Universidad de la Fuerzas Armadas ESPE, Sangolquí - Ecuador
eghuilca@espe.edu.ec
[b]Department of Biotechnology, Faculty of Biotechnology, Universidad de las Américas, Quito - Ecuador
slicto@udlanet.ec
[c]Sustainable Chemistry Laboratory, Faculty of Chemical Sciences, Universidad Central del Ecuador, Quito - Ecuador
ronnyronn@hotmail.com
*fwproaño@espe.edu.ec

ABSTRACT

Microalgae had a negative impact on the overall sensory quality. Psychrophilic microalgae live in extremely cold environments, their growth increases because they have enzymes in their structure that only adapt to temperatures below 0 ° C. For this reason, the Sustainable Chemistry Laboratory of the Central University of Ecuador, together with the Ecuadorian Antarctic Institute (INAE), made an expedition in the Greenwich, Roberts, Dee, Barrientos and Antarctic Towers where several microalgae consortia were collected, where 15 samples from Greenwich Island and Roberts were analyzed at 21 days at different temperatures, from which the genera Chlorella sp, Chlorococcum sp and Stichococcus sp. Subsequently, isolation was made in Petri dishes to obtain monoalgal cultures. Each of the isolated genera was massified in a volume of 5 mL until reaching a volume of 250 mL in modified M1 medium at a temperature of 4 ° C and 24 ° C, 5000 lux and a photoperiod of 12:12 hours. The Bligh & Dyer method was used for the extraction of lipids. The values of the lipid concentration showed that the genus Chlorella sp is the highest concentration with a value of 0.2802 mg / mL at 4 ° C and a value of 2.6704 mg / mL at 24 ° C on the 22nd day of its exponential phase in comparison with the genera *Chlorococcum* and *Stichococcus sp.*

Keywords:

Chlorella sp, Microalgae psychrophilic, Antarctica, Lipids, Bligh & Dyer method.

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INTRODUCTION

Psychrophilic microalgae are photosynthetic organisms that belong to the plant kingdom and adapt to temperatures of -5 ° C (Martínez, 2010). In Ecuador, studies of psychrophilic microorganisms have been conducted in the Chimborazo Glaciers (Correa, 2013). The psychrophilic microalgae collected in the Greenwich, Roberts, Dee, Barrientos and Torre islands of the Antarctic Archipelago have survived to the present, according to data from the Pedro Vicente Maldonado Antarctic Station, the type of genera identified in the microalgae consortium has increased by 2. in 2013 to 6 in 2014. There are 35 samples collected in total, of which 11 belong to Greenwich Island and correspond to the largest number of samples collected containing psychrophilic algae so far. The collections that also have a high abundance belong to the Greenwich Island with 5 genera. The psychrophilic microalgae that have less survival belong to Dee Island (Ecuador Antarctic, 2014). The Sustainable Chemistry Laboratory of the Central University of Ecuador is a government entity that aims to obtain different genera of microalgae from the samples collected, as well as the isolation of the genera: Chlorella sp, Chlorococcum sp and Stichococcus sp, to carry out processes at a pilot scale to subsequently extract lipids and synthesize biodiesel. The research focuses on the field and laboratory area to study the essential oil extracted from psychrophilic microalgae to make way for the synthesis of biofuel as an alternative source of diesel, as well as the isolation and massification of surviving microalgae (Flores, 2013). The obtaining of biofuel is extracted from lipids of microalgae collected at low temperatures belonging to the Chlorophyta, Bacillariophyta and Euglenophyta division (Amaro, Guedes, & Malcata, 2011). The most prevalent genera are Haematococcus sp., Chlorella sp., Stichococcus sp., Diatoma sp., Navicula sp. and Filamentosa sp, are characterized by being present in the three years of sampling (Molina, 2015). The marine and freshwater species that contain the highest amount of lipids for the production of biodiesel are Dunaliella with 116 mg / L / day and Nannochloris with 76.5 mg / L / day, as well as the genus Chlorella sp with 50 mg / L / day (MALGAS , 2013) . In our country, no psychrophilic microalgae genera have been reported for the synthesis of biodiesel (Flores, 2013). The open systems for obtaining microalgae were developed between 1935 and 1940 due to the scarcity of food. The application was made on an industrial scale with the purpose of obtaining an alternative source of proteins of vegetable and animal origin to replace food for human consumption (Colorado, Moreno, & Pérez, 2013). The first microalgae were massified to obtain lipids during the Second World War in Germany, the studies were carried out by German scientists with the purpose of purifying contaminated waters (MALGAS, 2013). In 1953, microalgae cultures are studied for the use of photosynthetic gas exchangers used in space travel and as sources of microbial proteins (BEAM, 2013). In 1980 the cultivation of microalgae is produced for the improvement of the environment, transforming the organic samples and sediments from the wastewater into biomass and renewed waters for later use in biofertilizers and water for irrigation. In 1982 at world level, oil increased value, giving alternative ideas such as the use of microalgae as a source of solar energy (Colorado, Moreno, & Pérez, 2013). From 2013 to 2015, samples of microalgae were collected in the islands near the Pedro Vicente Maldonado Scientific Station, climate diversity is an important factor in the synthesis of fatty acids to use them as an alternative source of energy. The sample collected at Punta Figueroa on Greenwich Island in Antarctica has guaranteed the best consortium of microalgae in the production of effective oils such as behemoth, myristic, stearic, palmitic, linoleidic, oleic, palmitoleic, arachidic and linoleaidal. The genus of microalgae and the temperature from which the sample was obtained is important for obtaining lipids in dry weight, as well as the extraction methods used (Flores, 2013).

Currently, the European Union has implemented ideas to develop energy as biodiesel from microalgae consortia with the purpose of

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reducing derivatives from oil sources and the emissions produced by gases during the greenhouse effect (Wackett, 2008). Among the genera of microalgae identified with higher lipid productivity are *Chlorella sp* and Chlorococcum sp (Flores, 2013) .But there are major drawbacks that researchers face with the use of microalgae: the presence of high concentrations of biomass means that more energy is required, and the moderate amount of biofuel produced by microalgae on a pilot scale (AINEnergía, 2015). The cryophyllous microorganisms commonly known as psychrophilic inhabit extremely cold environments, their growth increases because they have enzymes in their structure that only adapt to temperatures below 0 ° C. The cell membrane is made up of fats that flow to the point of freezing (Barreiro & Sandoval, 2006) . Among the psychrophilic Antarctic microalgae is *Chlorella sp*, which shows rapid growth in cell cultures (Chinnasamy & Bhatnagar, 2010). Its shape is spherical, green because it contains high levels of chlorophyll (Kanno & Kazie, 2005). Therefore, researchers have opted for studies with psychrophilic microalgae, which could represent a safe alternative for the production of lipids. Among the psychrophilic Antarctic microalgae investigated are mainly: Pandorina sp, *Chlorella sp*, *Stichococcus sp*, *Chlamydomona sp* and *Chlorococcum sp* (Molina, 2015). Other results obtained in microalgae such as *Scenedesmus ovalternus, Chlorella vulgaris, Nannochloropsis sp and Isochysis sp*, have determined the production of lipids with a growth of 32.7 mg / L / day, obtaining a large amount of biodiesel. These results allow us to conclude that some genera of microalgae could be useful for the production of lipids (Bérmudez, 2012).

MATERIALS AND METHODS

Collection of samples.

The samples were awarded by the Sustainable Chemistry Laboratory of the Central University of Ecuador as part of the project of the Ecuadorian Antarctic Institute (INAE). These were taken from Greenwich Island and Roberts Island located in Antarctica, later moved to Ecuador in different test tubes with their respective covers.

2.2. Adaptation process

Several samples of biomass were taken from the original tubes, where consortiums 15IGa2 with coordinates 35° 59′ 36′′S and 48° 27′ 27′′ O, consortium 15IGc1 with coordinates 35° 53′ 30′′S and 76° 43′ 17′′O and the 15IRe1 consortium with coordinates 35 ° 43′ 40′′ and 72° 13′ 27′′O were adapted to different temperatures in the laboratory after going through a 21-day adaptation process in function of the modified culture medium M1 as shown in Table 3.

Table 1

Adaptation of the genres of consortium 15IGa2, 15IGc1 and 15IRe1 at 21 days.

<table>
<tr><th>Consortium</th><th>Original Sample</th><th>Adaptation 4 ° C</th><th>Adaptation 24 ° C</th></tr>
<tr><td rowspan="5">15IGa2</td><td>Haematococcus sp.</td><td rowspan="5">Chlorella sp.
Chlorococcum sp.</td><td rowspan="5">They did not survive</td></tr>
<tr><td>Chlorococcum sp.</td></tr>
<tr><td>Chlorella sp.</td></tr>
<tr><td>Stichococcus sp.</td></tr>
<tr><td>Diatoma sp.</td></tr>
<tr><td rowspan="4">15IGc1</td><td>Chlorella sp.</td><td rowspan="4">Chlorella sp.
Stichococcus sp.
Chlorococcum sp.</td><td rowspan="4">They did not survive</td></tr>
<tr><td>Stichococcus sp.</td></tr>
<tr><td>Chlorococcum sp.</td></tr>
<tr><td>Chlorella sp.</td></tr>
<tr><td rowspan="4">15IRe1</td><td>Haematococcus sp.</td><td rowspan="4">Chlorococcum sp.
Chlorella sp.</td><td rowspan="4">Chlorococcum sp.
Chlorella sp.
Stichococcus sp.</td></tr>
<tr><td>Chlorococcum sp.</td></tr>
<tr><td>Chlorella sp.</td></tr>
<tr><td>Stichococcus sp.</td></tr>
</table>

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Isolation in solid culture medium.

This technique was used to separate the three selected microalgae genera after the adaptation process in this case were *Chlorella sp* belonging to consortium 15IGa2, Chlorococcum sp belonging to consortium 15IGe1 and Stichococcus sp belonging to consortium 15IRe1. Agar was added to the modified M1 medium and 40 mL were distributed in three Petri dishes, then two drops of each consortium were inoculated in each of the boxes and spread with a sterile bacteriological loop. The respective lid was placed and the box was covered with parafilm paper, the Petri boxes were inverted and placed at 24 ° C with a 24 hour photoperiod, illumination of 4500 lux for two weeks. The microalgae were observed in the inverted microscope.

Preparation of culture medium

Three test tubes were used with 5 mL of modified M1 medium and a small amount of each microalga isolated from its respective Petri box was placed with a 24 hour photoperiod at 24 ° C with 4500 lux illumination, placing a stopper on the part top of the tube so that it has aeration for two weeks. The culture medium used for the microalgal growth was the modified M1 medium.

Modified culture medium M1

The medium was prepared in three different bottles according to the amount of volume required, the volume of distilled water was measured with a 1000 mL pipette, then the three bottles were autoclaved for 90 minutes at a pressure of 15 PSI and a temperature of 121 ° C. The composition of the modified M1 medium is shown in Table 2 (Salas, 2015).

Table 2
Composition of modified culture medium M1 for 1000 mL (Salas, 2015)

STOCK	CONCENTRATION
Stock I	**(g / L)**
KH_2PO_4	0.3
K_2HPO_4	0.18
Stock II	**(g / L)**
$FeCl_3.6H_2O$	0.002
Stock III	**(g / L)**
NaCl	0.1
$CaCl_2$	0.02
$MgSO_4$	2.65
$FeSO_4$	0.002
KNO_3	0.7025
KCl	0.30
$ZnSO_4$	0.0007
H_3BO_3	0.034
EDTA	0.03
$MnSO_4$	0.003
$CoCl_2$	0.02 mg
$CuSO_4$	0.3
Na_2Mo	0.04 mg

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Conditions culture microalgae psychrophilic Antarctic

Once growth was obtained in each test tube, it was massed in 18 bottles of 250 mL. The genera *Chlorella sp, Chlorococcum sp and Stichococcus sp* were inoculated into the modified M1 medium in three bottles of 250 mL per each gender at temperatures of 4° C and 24° C. A system was added to each of the bottles. Aeration of 0.5 L / min, a fuse glass with a diameter of 0.6 mm was attached to a hose, this system was connected to a 5000 Power Pump of 60 Hz, 5 W and 110 V with 2 outputs. Lighting was installed using lamps at 5000 lux with a photoperiod of 12:12 hours. The jars are monitored every day. A cell count was performed using the Neubauer chamber every 3 days. A data record was kept for 28 days to obtain values in the graph of absorbance vs lipid concentration, using the Excel 2008 program.

Bligh & Dyer extraction method

Five mL of each vial with its respective microalga at different temperatures in 18 test tubes was placed, placing aluminum foil on top to avoid contamination, placed in the oven at 80 ° C in a period of 48 hours. After this time elapsed, 3 mL of a mixture of chloroform-methanol solvents (1: 2) was added. The test tubes (Tube 1) were sonicated in 3 cycles of 15 minutes by adding distilled water and ice. The tubes were incubated at 4 ° C for 24 hours protecting them from the light with aluminum foil. The next day the tubes were sonicated in 3 cycles of 15 minutes adding distilled water and ice. They were centrifuged at 4700 rpm for 20 minutes at 24 ° C, then the extracts were recovered with a Pasteur pipette, to transfer the supernatant to new 15 mL tubes (Tube 2). 1.5 mL of a chloroform methanol (1: 2) solvent mixture was added to the residual biomass of (Tube 1), this step is done to extract more dry biomass lipids from the microalgae. It was again centrifuged at 4700 rpm for 20 minutes at 24 ° C recovering more extract from (Tube 1) to place it in (Tube 2). 2 mL of distilled water from (Tube 2) containing the extract was added and vortexed. The excess water was removed with a Pasteur pipette, and the last centrifugation was performed at 4700 rpm for 10 minutes at 24 ° C to separate the lower phase formed by chloroform and lipids. Then 1 mL of chloroform was added to further separate the lower phase, a Pasteur pipette was introduced with great care, air was bubbled to the bottom of the tube. The phase of chloroform and lipids was passed from (Tube 2) to (Tube 3), and how much volume was taken was measured. The aqueous phase was washed with 1 mL of chloroform, mixed in the vortex and centrifuged at 4700 rpm for 10 minutes at 24 ° C, the lower phase was recovered and placed in (Tube 3). It was left in a water bath overnight with shaking at 37 ° C without a lid. The next day, after the chloroform was evaporated, 2 mL of concentrated sulfuric acid were added. The tubes were sealed with aluminum foil in order to avoid contamination, a blank was prepared (Tube with sulfuric acid). The tubes were heated in an oven at 200 ° C for 15 minutes without lids. The tubes were placed in the refrigerator at 4 ° C to cool, 3 mL of distilled water was added. The tubes were sonicated for 15 minutes and mixed with vortex until the sample was homogeneous and without residues of organic matter. The samples were transferred to the cells and the absorbance was read at 375 nm in the spectrophotometer. The materials used are shown in Table 3.

Table 3

Materials used in the Bligh & Dyer Method

Equipment	Reagents	Materials
	Test tubes	
Refrigerator	Aluminum foil	Distilled water
Sonicador	Glass bottles	Ice
Centrifuge	100 mL test tube	Chloroform
Vortex	Pipettes	Methanol
Digital balance	Petri boxes	Sulfuric acid
	Stove	

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Quantification of lipids extracted by spectrophotometry

The amount of lipids extracted from the genera *Chlorella sp, Chlorococcum sp Chlorella sp, Chlorococcum sp* and *Stichococcus sp* was obtained in the spectrophotometer. To perform the quantification, all the steps mentioned above were developed in the Bligh & Dyer extraction method to measure the absorbance at 375 nm. The concentration of lipids obtained from each gender at different temperature was calculated by the following equation:

$$[Lípids] = \left(\frac{Ab-0.0126}{2.8311}\right) x \frac{3.5}{Vext} \qquad \text{(Equation 1)}$$

Where: Ab (Absorbance), Vext (Volume extracted from the sample). The concentration of lipids indicated the lipid production in time. These data were calculated through the SPSS 15.0 program The equation of the line was obtained with experiments previously carried out at the Central University of Ecuador.

$$y = 2.831 + 0.0126 \qquad \text{(Equation 2)}$$

$R^2 = 0.999$

Determination of the growth curve of Stichococcus sp.

To obtain the dry weight of the genus Stichococcus sp. in the stationary phase to determine the growth curve, first the test tubes were weighed on the analytical balance before placing the volume of each bottle, then 5 mL of each bottle was placed at a temperature of 4 ° C and 24 ° C In the 6 test tubes measured above, aluminum foil was used in the upper part to avoid contamination in the sample, then the tubes were placed in the oven at 80 ° C in a period of 48 hours. Once the dry biomass was obtained inside each tube, it was reweighed in the analytical balance, the results were taken in a register and analyzed using the Excel 2008 program. Through the analysis of ANOVA and Duncan's Test, the factor temperature and gender in the production of lipids for the synthesis of biofuel was determined through the SPSS 15.0 program.

RESULTS AND DISCUSSIONS

Chlorella sp, Chlorococcum sp and Stichococcus sp.

Massification

Microalgae samples obtained from the 15IGa2, 15IGc1 and 15IRe1 consortiums of Greenwich Island and Roberts Island of the Antarctic Islands were conserved in 3 test tubes, with modified M1 medium, in a 24 hour photoperiod at a temperature of 24 ° C with aeration and illuminance of 4500 lux. After two weeks of isolation, the microalgae isolated in their respective Petri box were observed, and the genera *Chlorella sp, Chlorococcum sp and Stichococcus sp.* in the modified M1 medium with three repetitions of each in bottles of 250 mL at 4 ° C and 24 ° C in a photoperiod of 12:12 hours, with moderate aeration and with illuminance of 5000 lux.

Analysis of cell growth at different temperatures

The values indicated in Table 4 and Table 5 in relation to cell growth at the temperature of 4 ° C, indicated that in three replications (A, B and C), the average number of *Chlorella sp* cells per mL in the exponential phase on day 22 was 285›000 000 cel / mL, compared to the genus *Chlorococcum sp* which was 95›100 000 cel / mL, both microalgae were at the same temperature, phase, illumination, photoperiod and aeration.

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Table 4

Average cell growth of the genus Chlorella sp at 4 ° C

Chlorella / Ml				
DAYS	TO	B	C	AVERAGE
0	0	0	0	0
4	1,40E + 06	1.38E + 06	1.39E + 06	1.39E + 06
11	3,10E + 06	3.09E + 06	3,11E + 06	3,10E + 06
15	2,80E + 07	2,90E + 07	2,84E + 07	2,85E + 07
18	1,00E + 08	1.04E + 08	1.08E + 08	1.04E + 08
22	2,90E + 08	2.81E + 08	2,85E + 08	2,85E + 08
25	2,60E + 08	2,55E + 08	2.67E + 08	2.61E + 08

Table 5

Average cell growth of the genus Chlorococcum sp at 4 ° C

Chlorococcum / mL				
DAYS	TO	B	C	AVERAGE
0	0	0	0	0
4	4.67E + 05	4.60E + 05	4.63E + 05	4.63E + 05
11	1.03E + 06	1.03E + 06	1.04E + 06	1.03E + 06
15	9.33E + 06	9,67E + 06	9,47E + 06	9,49E + 06
18	3,33E + 07	3,47E + 07	3,60E + 07	3,47E + 07
22	9,67E + 07	9,37E + 07	9,50E + 07	9.51E + 07
25	8.67E + 07	8,50E + 07	8.90E + 07	8.69E + 07

The three repeats (A, B and C) of the genus *Stichococcus sp* at a temperature of 4 ° C indicated an average value of cells per gram on day 22 of its exponential phase of 0.88 cel / g, in this case performed the dry weight count, due to the fact that a cell count cannot be executed in the Neubauer Chamber due to its morphology. The values are shown in Table 6.

Table 6

Average cell growth of the genus Stichococcus sp at 4 ° C

Stichococcus / g				
DAYS	TO	B	C	AVERAGE
0	0	0	0	0
4	0.02	0.02	0.02	0.02
11	0.16	0.17	0.16	0.16
15	0.38	0.36	0.36	0.37
18	0.65	0.68	0.68	0.67
22	0.88	0.90	0.88	0.88
25	0.74	0.78	0.78	0.76

The data indicated in Table 7 and Table 8 in relation to cell growth at 24 ° C, showed that in three replications (D, E and F) the average cell number of *Chlorella sp* per mL in the exponential phase in the day 22 was 604,000,000 cel / mL compared to the genus *Chlorococcum sp* which was 201,000,000 cel / mL, both genera were in equal conditions.

Table 7

Average cell growth of the genus Chlorella sp at 24 ° C

Chlorella / mL				
DAYS	D	AND	F	AVERAGE
0	0	0	0	0
4	1,35E + 06	1.36E + 06	1.34E + 06	1,35E + 06
11	1.38E + 08	1,48E + 08	1,52E + 08	1,46E + 08
15	3.53E + 08	3.05E + 08	3,11E + 08	3,23E + 08
18	4.70E + 08	4,75E + 08	4,80E + 08	4,75E + 08
22	6,00E + 08	6.02E + 08	6,10E + 08	6.04E + 08
25	5,26E + 08	4.78E + 08	5,18E + 08	5.07E + 08

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Table 8

Average cell growth of the genus Chlorococcum sp at 24 ° C

DAYS	Chlorococcum / mL			
	D	AND	F	AVERAGE
0	0	0	0	0
4	4.50E + 05	4.53E + 05	4,47E + 05	4.50E + 05
11	4,60E + 07	4.94E + 07	5.05E + 07	4,86E + 07
15	1,18E + 08	1.02E + 08	1.04E + 08	1.08E + 08
18	1,57E + 08	1.58E + 08	1,60E + 08	1.58E + 08
22	2,00E + 08	2.01E + 08	2.03E + 08	2.01E + 08
25	1,75E + 08	1,59E + 08	1.73E + 08	1,69E + 08

The three repetitions (D, E and F) of the genus *Stichococcus sp* at 24 ° C presented an average value of cells for each gram on day 22 of its exponential phase of 1.18 cel/g, the cell count was performed by dry weight. The values are indicated in Table 9.

Table 9

Average cell growth of the genus Stichococcus sp at 4 ° C

	Stichococcus / g			
DAYS	D	AND	F	AVERAGE
0	0	0	0	0
4	0.13	0.15	0.15	0.14
eleven	0.30	0.30	0.29	0.30
fifteen	0.60	0.63	0.63	0.62
18	0.80	0.83	0.80	0.81
22	1,15	1,18	1.20	1,18
25	0.85	0.78	0.78	0.80

The genera *Chlorella sp, Chlorococcum sp and Stichococcus sp* grew in the modified M1 medium that contained several macroelements and microelements to contribute to microalgal growth (Salas, 2015) . Figure 8 and Figure 9 show that *Chlorella sp* microalgae had greater cell growth at 28 days in its three repetitions at 4 ° C and 24 ° C in relation to the genus *Chlorococcum sp* indicated in Figure 1 and Figure 2.

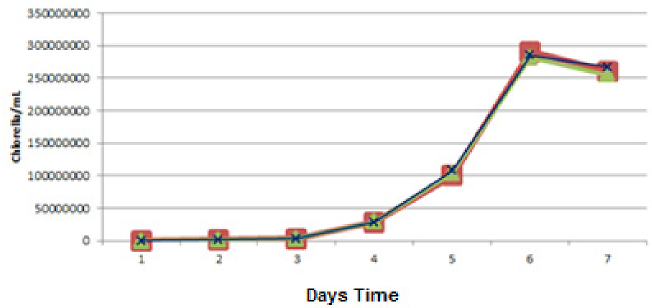

Figure 1. Growth curve of *Chlorella sp* cultivated in M1 medium modified in 3 repetitions at 4 ° C

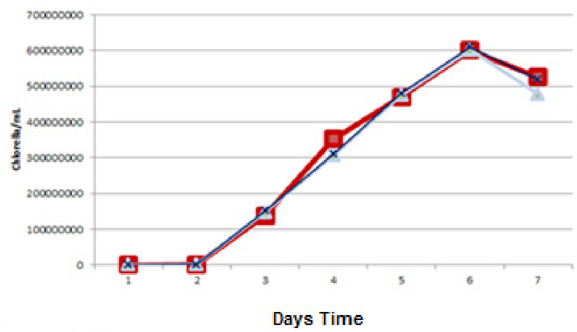

Figure 2 Growth curve of *Chlorella sp* cultivated in M1 medium modified in 3 repetitions at 24 ° C

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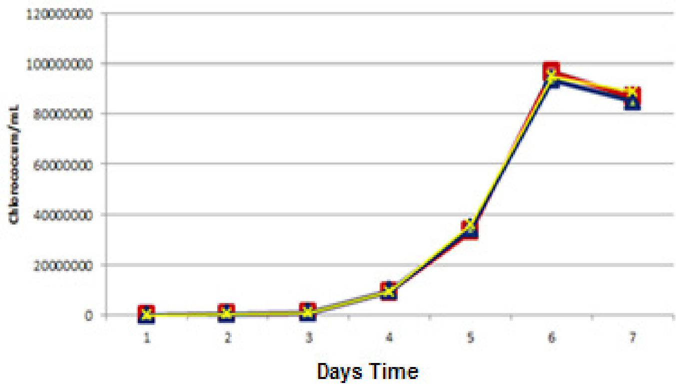

Figure 3. Growth curve of *Chlorella spcultivated* in M1 medium modified in 3 repetitions at 4 ° C

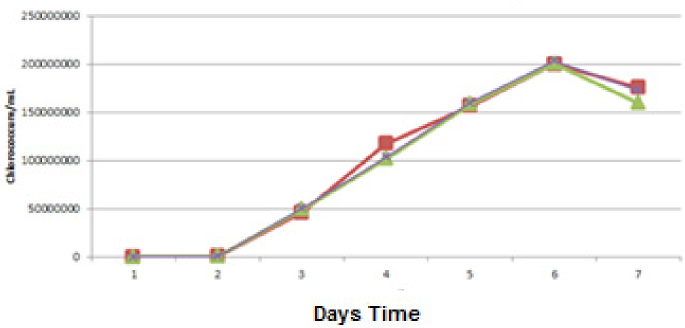

Figure 4. Growth curve of *Chlorococcum sp* cultivated in M1 medium modified in 3 repetitions at 24 ° C

Figure 3 and Figure 4 show the cell growth of Stichococcus sp at 28 days, with three repetitions at different temperatures. This microalga was under the same conditions as *Chlorella sp and Chlorococcum sp*.

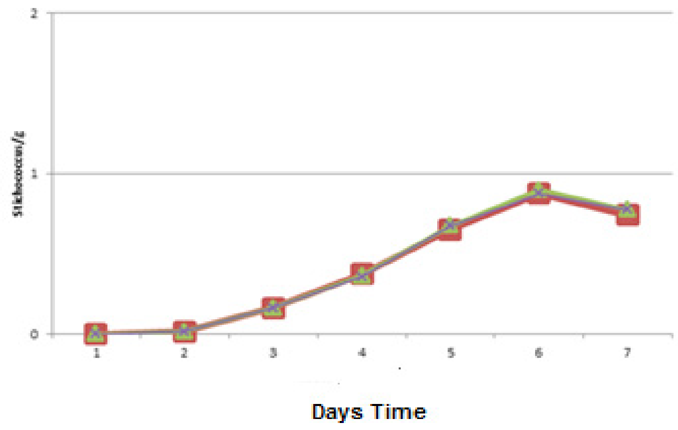

Figure 5. Growth curve of *Stichococcus sp* cultivated in M1 medium modified in 3 repetitions at 4 ° C

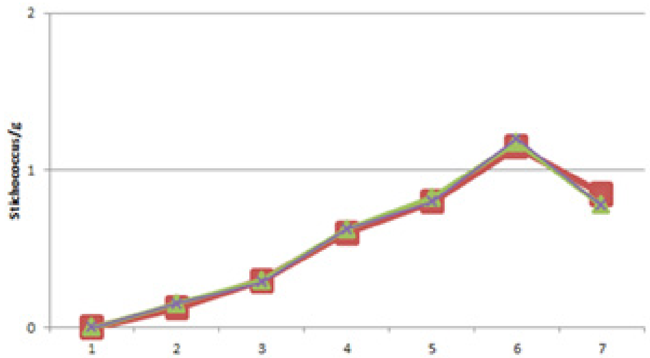

Figure 6. Growth curve of *Stichococcus sp* cultivated in M1 medium modified in 3 repetitions at 24 ° C

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The genus *Chlorella sp* showed greater cellular growth until day 22 of its exponential phase in comparison with the genera *Chlorococcum sp and Stichococcus sp* , because it has a better capacity of absorption of CO2, which is why it captured all the nutrients contained in the culture medium used which in this case was the modified M1 (Khotimchenko & Yakolevka, 2005). The time in which the microalgal cells were generated is optimal for the production of lipids and subsequently sintering biofuel and several metabolites. It should also be noted that the modified culture medium M1 favors the growth of the microalga due to the nitrogen and phosphorus components in its composition (Fernández, Sánchez, & Molina, 2001). The cell growth of *Chlorella sp* can be identified in the color according to the light that the microalga receives. At a temperature of 4 ° C they showed a dark green coloration, this is given by the cianelles that they have inside. As the bottles preserved at 24 ° C they took a light green tone, after 7 days they suffer a deterioration by the photo-oxidative effect since the light and the external oxygen act as secondary factors on the microalga, so that more volume of the modified medium M1 was placed, this causes the cells to acquire again the nutrients that were administered at the beginning and thus be able to assimilate components that have not yet been captured by the genus (Chisti, 2007). The modified M1 medium was not used by the *Chlorococcum sp and Stichococcus sp* for cell growth. One of the factors that influences is the composition that the medium possesses, for this reason other different culture media must be tested until finding the appropriate one in future projects to carry out cell growth and later the extraction of lipids (Jaramillo, 2011). The BBM culture medium allows the development of a large number of cells of *Chlorococcum sp* and *Stichococcus sp* although it presents a small amount of nitrogen in its composition (Kuma, 2010).

Measurement of extracted lipids

The data obtained in Table 12 in relation to the extraction of lipids of the genera *Chlorella sp, Chlorococcum sp and Stichococcus sp*, indicated that the lipid concentration of the genus *Chlorella sp* at a temperature of 4 ° C on day 22 of the exponential phase it was 0.2802 mg / mL in comparison with the genus *Chlorococcum sp* which presented a concentration of 0.0922 mg / mL and the genus *Stichococcus sp* which was 0.0685 mg / mL. All genera were at the same conditions of aeration, light and photoperiod. Likewise, the concentration of lipids extracted at a temperature of 24 ° C was greater in the genus *Chlorella sp* on day 22 of the exponential phase with a value of 2.6702 mg / mL, while in the genus *Chlorococcum sp and Stichococcus sp* , the concentration was 0.8890 mg / mL and 0.5595 mg / mL respectively, as shown in Table 11.

Table 10

Lipid concentration of microalgae genera at 4 ° C

Days	Lipids Chlorella sp [mg / mL]	Lipids Chlorococcum sp [mg / mL]	Lipid Stichococcus sp [mg / mL]
0	0	0	0
4	0,1167	0,0380	0.0177
11	0,1682	0,0549	0,0445
15	0.1961	0.0642	0,0519
18	0,2130	0,0698	0.0551
22	0.2802	0,0922	0,0685
25	0.2403	0,0789	0,0587

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Table 11

Lipid concentration of the microalgae genera at 24 ° C

Days	Lipids Chlorella sp [mg / mL]	Lipids Chlorococcum sp [mg / mL]	Lipid Stichococcus sp [mg / mL]
0	0	0	0
4	0,0740	0,0238	0,0180
11	0,2331	0,0765	0.0653
15	0,2765	0,0910	0,0671
18	1,2562	0.4175	0,1785
22	2,6704	0.8890	0.5595
25	1,7097	0,5687	0.4309

Figure 7 and Figure 8 show the average of lipid concentrations at 28 days, with three repetitions at a temperature of 4 ° C and 24 ° C.

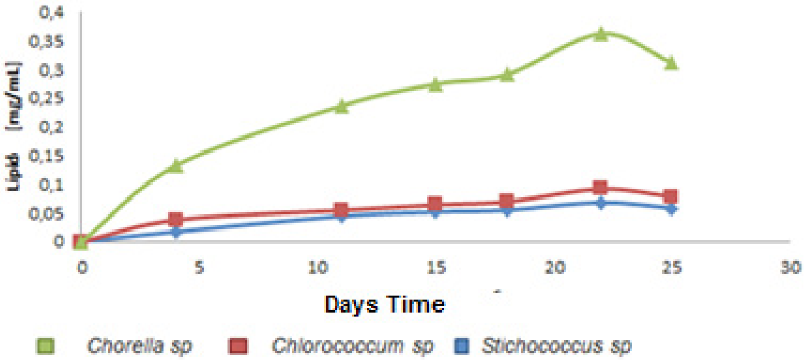

Figure 7. Average concentration of lipids of *Chlorella sp, Chlorococcum sp and Stichococcus sp* at 4 ° C

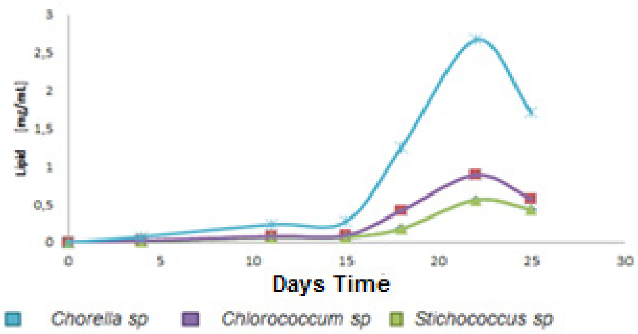

Figure 8. Average concentration of lipids of *Chlorella sp, Chlorococcum sp and Stichococcus sp* at 24 ° C

Chlorella sp adapted at 4 ° C showed higher concentration of lipids compared to *Chlorococcum sp and Stichococcus sp*. The lower temperatures increased the level of unsaturation and the light intensity of 5000 lux favored the microalgae in the accumulation of triglycerides with a high saturation level (Anderson & Katja, 2003). In addition, the genus *Chlorella* sp at 24 ° C showed higher lipid production due to the capture of solar energy and CO2 from the environment. This microalgae is one of the genera with the highest percentage of lipids, the *Chlorella emersonii* species produces 63% in comparison with the species *Chlorococcum oleofaciens* that presents 44.3% (Faife, Otero, & Álvarez, 2012). *Chlorella sp* presents higher productivity of lipids at different temperatures due to the high level of triglycerides that contains, in addition to the genetic constitution, the light intensity, pH, salinity, minerals that were factors that influenced the lipid production (Lee, Lewis, & Ahsman , 2009). In all three genera studied there is a variation in the lipid content. The genera *Chlorococcum sp and Stichococcus sp* showed very low lipid levels because their cell growth could occur under unfavorable conditions, there were not enough stress conditions and they did not assimilate all the nutrients

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of the culture medium administered at both temperatures (Liang, Dong, Miao, & Dai, 2006). The optimal culture medium for these microalgae genera is the BBM or Bristol because it contains a stock solution of macronutrients, a stock of micronutrients and a stock of traces. In addition, the pH of the medium, the concentrations of each of the stocks, the nitrogen composition, as well as the external factors for the development of cellular growth of microalgae should be taken into consideration (MALGAS, 2013) . The genera *Chlorella sp and Chlorococcum sp and Stichococcus sp* presented lipid production at different temperatures, these results were obtained thanks to the Bligh & Dyer method, which allows to extract a large amount of fatty acids using methanol and chloroform as solvents, without the use of heat (Salas, 2015) The analysis of variance was performed for the cell growth of *Chlorella sp and Chlorococcum sp* at different temperatures, where these hypotheses were verified. The analysis of variance was carried out for the lipid concentration of the genera *Chlorella sp, Chlorococcum sp and Stichococcus sp* at different temperatures, where these hypotheses were verified.

Ho: Microalgae gender effect = 0

Ha: Microalga genre effect ≠ 0

Ho: Temperature effect = 0

Ha: Temperature effect ≠ 0

By means of the analysis of variance or ANOVA in the SPPS 15.0 program as shown in Table 1 3 , it was verified that the meanings given by the microalgae and temperature gender factor, related to the significance of 0.05 are lower, so that proceeds to reject the null hypotheses of equality in the factors. For this reason, it was analyzed that the *Chlorella sp* microalgae had a higher lipid concentration at different temperatures.

Table 12

Analysis of ANOVA or variance of the genera Chlorella sp, Chlorococcum sp Stichococcus sp at different temperatures.

Variance analysis					
Source	Sum of Squares Type III	gl	Half quadratic	F	P
Model	4,054	5	0.811	3,935	0.0001
Genus	1,647	2	0.823	3,996	0.0001
Temperature	1,646	1	1,646	7,987	0.0001
Error	6,181	30	0.206		
Total	13,917	36			

Note: *To accept the null hypothesis P> 0.05. F = Ratio of mean squares, P = significance*

The Duncan Test was analyzed through the SPSS 15.0 program to check the genus of microalgae that had the highest lipid production taking into consideration the temperature at which each one of them was. In Table 13 the analysis of the gender factor was carried out, resulting in the *Chlorella sp* microalga indicating a mean value of 0.619, the value is high so it was grouped in a subset, while the genus *Chlorococcum sp* showed a mean value of 0.205 and the genus *Stichococcus sp* had a mean value of 0.134, presenting low levels for this reason were grouped in the same subset.

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Table 13

Duncan test of the genera Chlorella sp, Chlorococcum sp Stichococcus sp at different temperatures.

Duncan Alpha test = 0.05				
Error: 0.206	gl: 30			
Gender	Socks	N	Subset	
Chlorella sp	0.619	12	1	
Chlorococcum sp	0.205	12		2
Stichococcus sp	0,134	12		2

CONCLUSIONS

All psychrophilic microalgae present in glaciers in Antarctica produce lipids for the synthesis of biofuel, the genus *Chlorella sp* being the highest concentration with a value of 0.2802 mg / mL at a temperature of 4 ° C and a value of 2 , 6704 mg / mL at a temperature of 24 ° C on day 22 of its exponential phase. The analysis of the lipid composition shows that the temperature and composition of the modified M1 culture medium are important for the adaptation of the microalgal cells, when these are at temperatures of 4 ° C and 24 ° C, of which the samples adapted to room temperature showed greater cell growth. All samples of microalgae obtained from the 15IGa2, 15IGc1 and 15IRe1 consortiums of Greenwich Island and Roberts Island of the Antarctic Islands were initially massed at a volume of 5 mL, from which the genera *Chlorella sp*, Chlorococcum sp and Stichococcus sp were isolated. until reaching a volume of 250 mL, in a 24-hour photoperiod with aeration and illuminance of 4500 lux. The extraction of lipids was carried out from the Antarctic psychrophilic microalgae using the Bligh & Dyer method for the synthesis of biofuel. The genus *Chlorella sp* generates more lipids at a temperature of 24 ° C, and in a small amount at a temperature of 4 ° C. The genera Chlorococcum sp and Stichococcus sp generated a low amount of lipids at different temperatures, taking into account that the lipid concentration is low, it is not ruled out that other factors have influenced the production of lipids.

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